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The Goodyear Tire & Rubber Company

Akron, Ohio 44316 - 0001

September 25, 1998

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Office of Pollution Prevention and Toxics (OPPT)
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Washington, D.C. 20460

Confidential No CBI

Dear Ladies/Gentlemen:

Subject: TSCA Section 8(e) Notice

This submittal does not contain Confidential Business Information.

On September 9, 1998, The Goodyear Tire & Rubber Company received a copy of a draft final report of a four-week dietary study performed by American Health Foundation. The current identity of the test material is as follows:

CAS Name: Propanoic acid, 3-(dodecylthio)-, oxybis(2,1-ethanediyoxy-2,1-ethanediyl) ester

CAS Number: 64253-30-1

The preliminary results (that may indicate a substantial risk) indicate that after 28 days, the high dose (1900 ppm) induces in both sexes a reduction in intercellular glucose availability and utilization, protein catabolism, as well as inadequate absorption of iron. These lead to ketonuria, hypoproteinemia, hypolipidemia, hyposideremia, increased BUN, dehydration, hypovolemia, and hypoxia in both hepatic and extrahepatic tissues. Further, these changes result in overload of the cardiovascular system without evidence of renal failure and increases in hepatocellular and urothelial cell proliferation. During recovery, there is improvement, but not complete reversal of these findings. Consequently, under the requirements of Section 8(e) of the Toxic Substances Control Act and EPA's Statement on Interpretation and Enforcement Policy, 43 Fed. Reg. 1110 (16 March 1978), The Goodyear Tire & Rubber Company is providing the EPA with a copy of the draft final report Four-week Dietary Study of WINGSTAY SN-1 in Fischer 344 Rats.

These findings have not been reported in final form. However, upon completion, the final report will be forwarded to EPA.

My address and telephone number are as follows:

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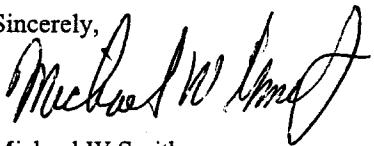
8EHQ-98-14281



88980000248

The Goodyear Tire & Rubber Company
Department 100D
1144 East Market Street
Akron, Ohio 44316-0001
Telephone: (330) 796-2362

Sincerely,



Michael W Smith
Section Manager, Chemical Information
Systems & Regulatory Affairs

attachment

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DRAFT FINAL REPORT

Study Title:

Four-Week Dietary Study of Wingstay ®SN-1 in Fischer 344 Rats

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98 SEP 30 PM 3:41

Sponsor:

THE GOODYEAR TIRE & RUBBER COMPANY
Toxicology and Regulatory Compliance
Research Division
142 Goodyear Boulevard
Akron, Ohio 44305-0001

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Testing Facility:

American Health Foundation
One Dana Road
Valhalla, NY 10595
U.S.A.

Laboratory Study Identification:

AHF Study R-1777

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One Dana Road
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COMPLIANCE STATEMENT

Study Title: Four-Week Dietary Study of Wingstay®SN-1 in Fischer 344 Rats

This study was conducted in compliance with the Good Laboratory Practice Standards of the USEPA Regulations, Part 792, 40 CFR, Final Rule, August 17, 1989, pp. Fed. Reg. 54, Nr. 158, pp 34034-34052 and with any applicable amendments, as well as the GLP Principles of the OECD (1981) and Japanese Ministry of International Trade and Industry (1993). All deviations from the protocol are given in Appendix 21. There were no known deviations from the aforementioned regulations that would impact on the interpretation of the results in this report. Even Deviation No. 2 did not interfere with the results because of consistent and significant functional and morphologic changes observed in high exposure animals of both genders.

Study Director:

M. J. Iatropoulos, MD, PhD.

Date

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QUALITY ASSURANCE UNIT STATEMENT

Study Number: R-1777

Study Title: Four-Week Dietary Study of Wingstay® SN-1
in Fischer 344 Rats

Dates of Inspections and Audits

01/14/97, 01/21/97, 01/22/97

02/05/97

02/11/98 - 02/17/98,

03/02/98 - 03/06/98

Dates of Reports

01/22/97

03/05/97

03/09/98

This signed statement indicates that the AHF Quality Assurance Unit has monitored this study and reviewed the data and final report as required by the EPA Good Laboratory Practice Standards and other equivalent regulations. The reviews indicate that the final report accurately presents raw data as developed during the study.

Henry E. Prescott, PhD
Quality Assurance Unit

Date

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STUDY IDENTIFICATION

Four-Week Dietary Study of Wingstay ® SN-1 in Fischer 344 Rats

Abbreviated Study Title: Four-Week Study of SN-1 in Rats

AHF Study Number: R-1777

Test Substance: WINGSTAY ® SN-1
1:11 (3, 6, 9-trioxaundecyl)bis-(dodecylthio)propionate

Sponsor/Study Monitor: The Goodyear Tire & Rubber Company
Toxicology and Regulatory Compliance
Research Division
142 Goodyear Boulevard
Akron, Ohio 44305-0001
Dr. Richard Serva

Study Time Table:

- a. First day of exposure: December 24, 1996 (males)
December 31, 1996 (females)
- b. 29-30-Day Termination (Sacrifice): January 22-23, 1997 (males)
January 29-30, 1997 (females)
- c. 43-Day Termination (Sacrifice): February 5, 1997 (males)
February 12, 1997 (females)
- d. Draft Final Reports: January 21, April 21, and September 10, 1998
- e. Final Report:

Study Director: Michael J. Iatropoulos, M.D., Ph.D.

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Analytical Toxicology Lab (ATL): Klaus D. Brunnemann, M.S.

Statistical Support: Brian P. Pittmann, M.S.

Data Collection: James Hosey, M.S.

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SUMMARY

Wingstay ®SN-1 (SN-1), 1:11 (3,6,9-trioxaudecyl)-bis-(dodecylthio) propionate, a Goodyear product, which is used as a secondary antioxidant, was given daily in the diet to 44 male and 44 female Fischer 344 (F344) rats for 28 consecutive days, followed by a 14-day exposure-free period of recovery. SN-1 was mixed in powdered NIH-07 diet to achieve a concentration of 0.012% (120 ppm), set to deliver 7.5 mg/kg bw/day; or 0.047% (470 ppm), set to deliver 30 mg/kg/day; or 0.19% (1900 ppm), set to deliver 120 mg/kg/day.

Clinical observations, body weights and food consumption were monitored. At terminations, after 28 and 42 days, hematology, clinical chemistry and urinalysis were conducted. In addition, the red cell distribution width (RDW), serum iron, unsaturated iron binding capacity (UIBC), total iron binding capacity (TIBC), transferrin (TF) and erythropoietin (EPO) were also measured. Complete necropsies were performed, and the absolute and relative weights for the following tissues were recorded: liver, kidneys, pituitary, heart, spleen, thyroids, adrenals, brain, uterus, ovaries and testes. All sampled tissues (33 in males and 34 in females) were processed and H&E slides were subjected to microscopic evaluation. In addition, slides from liver and urinary bladder were also subjected to immunohistochemical staining for proliferating cell nuclear antigen (PCNA), and the labeling indices of these tissues were determined by scoring of stained nuclei.

No compound-related clinical findings occurred. After 28 days, in the high dose groups of both genders, achieved body weight gain at termination was reduced by 7.2% in males and 4.5%

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in females. At the end of 42 days, achieved body weight gain reduction recovered in females and was improved in males. In the same groups, food consumption was decreased at 28 days in both genders. At 29 days, there were significant high dose changes in the following parameters: body weight (males), relative liver (both genders), relative kidney (males), relative thyroid (males), relative heart (males), relative adrenal (males), relative brain (males), relative testicular and relative ovarian weights (not significant), MCV (females), MCHC (males), MCH (males), prothrombin time (both genders), APTT (females), total proteins (males), cholesterol (both genders), triglycerides (males), LAP (females), serum iron (both genders), TIBC (males), UIBC (females), transferrin (males), blood chlorides (males), alkaline phosphatase (females), blood glucose (not significant, both genders), BUN (both genders), urine pH (females), urine ketones (both genders), liver proliferation index (both genders) and urinary bladder proliferation index (both genders).

Based on these statistically significant and other biologically significant changes, it can be assumed that the high exposure of SN-1 causes reduction in intracellular glucose availability and protein catabolism with ketonuria, hypoglycemia, increased protein catabolism, hypoproteinemia, increased BUN, (two higher doses in both genders), hypocholesterolemia, prolongation of prothrombin time with shortening of APTT, hypovolemia, dehydration, reduction of body weight gain, interference with iron homeostasis resulting in hyposideremia (all three exposures in males and two higher exposures in females), decreased red cell mass, increased mean corpuscular hemoglobin concentration (males), increases in relative liver, kidney and heart weights, increases in hepatocellular apoptosis (both genders), increases in hepatocellular (both genders) and urothelial cell (high dose in

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males and two higher doses in females) proliferation indices.

In conclusion, under the conditions of this study, SN-1 at 1900 ppm induces in both genders a reduction in intracellular glucose availability and utilization, protein catabolism, as well as inadequate absorption of iron. These lead to ketonuria, hypoproteinemia, hypolipidemia, hyposideremia, increased BUN, dehydration, hypovolemia and hypoxia in both hepatic and extrahepatic tissues. Moreover, these changes result in overload of the cardiovascular system without evidence of renal failure and increases in hepatocellular and urothelial cell proliferation. During recovery, there is improvement, but not complete reversal of these findings.

Summary Table - Males

Parameter	Terminations (Days)		HGB (g/dl)		HCT (%)		MCV (fl)		MCHC (%)		RDW (%)		PROTIME (seconds)		APTT (seconds)	
Group Identification	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43
Control	291	307	15.8	15.6	46.9	44.9	20.1	19.6	59.6	56.6	33.7	34.7	26.7	21.4	15.0	16.0
SN-1 120 ppm	294	-	15.4	-	44.9	-	20.8	-	60.6	-	34.4	-	26.7	-	15.5	-
SN-1 470 ppm	294	-	16.4	-	47.5	-	20.6	-	59.8	-	34.5	-	26.2	-	15.5	-
SN-1 1900 ppm	270*	292	16.1	16.6**	44.9	47.6**	21.3**	20.1*	59.4	57.8**	35.8***	34.8	26.0	24.4*	18.1*	15.5
Parameter	Terminations (Days)		Total Protein (g/dl)		Albumin (g/dl)		Globulin (g/dl)		Chol (mg/dl)		Triglyc (mg/dl)		LAP (units/l)		Iron (μg/dl)	
Group Identification	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43
Control	7.4	7.4	4.7	4.8	2.7	2.6	55.7	67.3	117.7	127.5	51.6	47.6	126	111	51.1	50.2
SN-1 120 Ppm	7.2	-	4.6	-	2.6	-	52.7	-	79.8*	-	46.6	-	90*	-	48.7	-
SN-1 470 Ppm	-	7.4	-	4.6	-	2.8	-	51.7	-	76.8*	-	44.1	-	53***	-	
SN-1 1900 Ppm	6.9***	7.1	4.5*	4.7	2.4*	2.4*	32.0***	44.3**	68.8**	83.8*	51.2	48.9	50***	59***	45.5**	45.6*
Parameter	Terminations (Days)		Cl (meq/l)		Ca (mg/dl)		P (mg/dl)		ALP (units/l)		Glu (mg/dl)		BUN (mg/dl)		Urine pH	
Group Identification	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43
Control	97.7	95.0	12.1	11.4	11.1	8.6	185	145	223	134	14.5	14.7	6.7	6.7	5.0	11.7
SN-1 120 ppm	99.5	-	11.7	-	10.6	-	183	-	134*	-	14.5	-	6.5	-	5.0	-
SN-1 470 ppm	100.2*	-	11.6	-	11.5	-	202	-	156	-	18.2*	-	6.3	-	8.3	-
SN-1 1900 ppm	101.3***	98.2*	11.1**	11.4	10.7	9.7*	204	162	140	170*	23.0***	20.8***	6.3	6.8	31.7**	40.0**
Parameter	Terminations (Days)		Rel Kid Wt (%)		Rel Thy Wt (%)		Rel Heart Wt (%)		Rel Adr Wt (%)		Rel Brain Wt (%)		Rel Testes Wt (%)		Liver Pl (%)	
Group Identification	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43
Control	2.99	2.97	0.73	0.70	0.0064	0.0056	0.33	0.35	0.020	0.019	0.67	0.64	1.35	1.29	4.1	4.3
SN-1 120 ppm	2.96	-	0.72	-	0.0045***	-	0.35	-	0.017	-	0.66	-	1.39	-	4.4	-
SN-1 470 ppm	3.09	-	0.71	-	0.0047***	-	0.33	-	0.022	-	0.66	-	1.34	-	4.5	-
SN-1 1900 ppm	3.64***	3.22***	0.79*	0.74*	0.0054*	0.0053	0.37**	0.34	0.026**	0.021	0.75**	0.67	1.49**	1.34	5.7***	4.4

0. femtoliter (a billionth of a microliter, 10^{-15} liter); pg, picogram (a trillionth of a gram, 10^{-12} gram); dl, deciliter; meq, milliequivalent; *, statistically significant from control at $p<0.05$; **, *** , p<0.01; ****, p<0.001; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; RDW, red cell distribution width; PROTIME, prothrombin time; APTT, activated partial thromboplastin time; Chol, cholesterol; Triglyc, triglycerides; LAP, leucine aminopeptidase; TIBC, total iron binding capacity; UIBC, total iron binding capacity; T-FER, transferrin; Cl, chloride; Ca, calcium; P, Phosphorus; ALP, alkaline phosphatase; Glu, glucose; BUN, blood urea nitrogen; pH, measure of hydrogen ion activity; Rel Kid Wt, relative kidney weight; Rel Thy Wt, relative thyroid weight; Rel Heart Wt, relative heart weight; Rel Adr Wt, relative adrenal weight; Rel Brain Wt, relative brain weight; Rel Testes Wt, relative testes weight; Liver Pl, liver proliferation index; Rel Kid Wt, relative kidney weight; -, not applicable.

Summary Table - Females

Parameter	Terminations (Days)		Body Weights (g)		HGB (g/dl)		HCT (%)		MCH (pg)		MCV (fl)		MCHC (%)		RDW (%)		PROTIME (seconds)		APTT (seconds)	
Group Identification	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43
Control	175	177	16.1	15.7	45.7	46.4	21.8	20.7	62.1	61.2	35.2	33.8	22.1	15.9	12.6	15.8	40.8	36.8		
SN-1 120 ppm	174	-	15.7	-	44.0	-	22.1	-	61.9	-	35.6	-	20.2	-	14.3	-	35.6	-		
SN-1 470 ppm	174	-	16.0	-	44.8	-	21.9	-	61.5	-	35.7	-	18.0	-	16.4*	-	28.9*	-		
SN-1 1900 ppm	167	173	15.8	15.9	43.9	45.1	21.8	21.1	60.5**	60.1**	36.0	35.2**	18.0	17.7	19.1***	12.6*	22.7***	28.5*		
Parameter	Terminations (Days)		Total Protein (g/dl)		Albumin (g/dl)		Globulin (g/dl)		Chol (mg/dl)		Triglyc (mg/dl)		LAP (units/l)		Iron (ug/dl)		TIBC (ug/dl)		UIBC (ug/dl)	
Group Identification	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43
Control	7.0	7.3	4.8	4.9	2.2	2.4	79.5	82.0	63.8	61.2	50.2	54.9	261	266	442	447	181	180	345	343
SN-1 120 ppm	6.8	-	4.6	-	2.2	-	74.7	-	50.3	-	49.8	-	267	-	434	-	168	-	349	-
SN-1 470 ppm	6.7	-	4.5	-	2.2	-	62.5**	-	47.5	-	49.8	-	136**	-	401*	-	265	-	335	-
SN-1 1900 ppm	6.6	6.8	4.6	4.6	2.0	2.2	48.5***	64.8*	79.2	68.8	54.2**	52.4	136**	113**	423	432	288*	319*	343	354
Parameter	Terminations (Days)		Cl (meq/l)		Ca (mg/dl)		P (mg/dl)		ALP (units/l)		Glu (mg/dl)		BUN (mg/dl)		Urine pH		Urine Ketones (mg/dl)		Urine PI (%)	
Group Identification	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43
Control	104.2	100.0	11.2	11.4	10.9	8.3	143	115	156	143	13.0	15.0	6.7	6.3	0.0	0.0	0.0	0.0		
SN-1 120 ppm	104.0	-	11.0	-	9.9	-	156	-	123*	-	14.0	-	6.3	-	0.0	-	0.0	-		
SN-1 470 ppm	106.2	-	11.1	-	10.7	-	165	-	130	-	16.7*	-	6.0*	-	3.3	-	3.3	-		
SN-1 1900 ppm	106.5	101.7	11.2	10.9	11.7	10.2**	197***	158**	134	130	20.5***	22.2***	6.0*	6.0	31.7*	6.7				
Parameter	Terminations (Days)		Rel Liver Wt (%)		Rel Kid Wt (%)		Rel Thy Wt (%)		Rel Adr Wt (%)		Rel Brain Wt (%)		Rel Ov Wt (%)		Liver PI (%)		UB PI (%)		UB PI (%)	
Group Identification	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43	29	43
Control	2.78	* 2.59	0.79	0.71	0.0071	0.0077	0.38	0.35	0.039	0.036	1.07	1.01	0.075	0.052	3.5	4.2	12.6	10.5		
SN-1 120 ppm	2.74	-	0.78	-	0.0067	-	0.40	-	0.034	-	1.09	-	0.057*	-	3.7	-	12.9	-		
SN-1 470 ppm	2.83	-	0.74	-	0.0065	-	0.40	-	0.038	-	1.06	-	0.058*	-	3.7	-	17.9**	-		
SN-1 1900 ppm	3.46***	3.00***	0.80	0.76*	0.0072	0.0069	0.39	0.38	0.036	0.035	1.08	1.02	0.061	0.053	5.5***	5.4***	17.5**	14.8*		

fl, femtoliter (a billionth of a microliter, 10^{-12} liter); pg, picogram (a trillionth of a gram, 10^{-12} gram); dl, deciliter; meq, milliequivalent; *, statistically significant from control at $p<0.05$; **, $p<0.01$; ***, $p<0.001$; prothrombin time; APTT, activated partial thromboplastin time; Chol, cholesterol; Triglyc, triglycerides; LAP, lecine aminopeptidase; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity; Adr, adrenal; Ov, ovary; PI, proliferation index, represents the % of PCNA positive nuclei out of the total number of nuclei counted; UB, urinary bladder; -, not applicable.

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INTRODUCTION

Wingstay ® SN-1 (SN-1) is a Goodyear product used as a secondary antioxidant. The SN-1 exposures used in this study were selected on the basis of previous (4-week) studies with Wingstay ® 100 and 200 products in Fischer 344 (F344) rats. In the 4-week study, with W100 at 1900 ppm, reduction in body weight and food consumption, a macrocytic anemia, increased liver weights and intraluminal changes in the urinary bladder were evident. With W200, at 1900 ppm, a macrocytic anemia, growth rate reduction, disruption of bilirubin metabolism, reduction of plasma osmotic pressure, and cardiovascular overload were present.

The purpose of this study was to assess the subchronic toxicity of SN-1 to male and female F344 rats, when administered daily in the diet for a period of four weeks, and followed by a 2-week exposure-free period of recovery. The information obtained from this study will be utilized to determine the doses of the test substance to be administered in the diet to rats chronically.

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TEST ANIMALS AND HUSBANDRY

Forty-four male and 44 female Fischer 344 (F344/N Tacf BR MPF) rats from Taconic Farms, Germantown, NY, were used in this study. They were approximately eight weeks of age on arrival. Upon receipt at the American Health Foundation (AHF), the animals were acclimated to laboratory conditions for approximately two weeks before dosing. During this time, they were screened for standard rodent pathogens and were released on December 24 and 31, 1996 for the males and females, respectively. They were also assigned to groups based on body weight (AHF SOP-090), and were identified by ear notch and toe clip (AHF SOP-150) (Text Table 1).

The animals were housed two per cage in solid floor polycarbonate cages, on irradiated corn cob bedding in a temperature ($72 \pm 8^{\circ}\text{F}$) and humidity ($55 \pm 20\%$) controlled room. The bedding was obtained from Andersons Management Corporation, Maumee, OH. Fifteen to twenty fresh air changes per hour were provided to the room and 12 hours of continuous fluorescent lighting (25-30 ft candles) were provided daily.

Powdered NIH-07 diet, as 5018 NIH meal (PMI Feeds, Inc., St. Louis, MO), and tap water were available *ad libitum*. The water was supplied by the Westchester County (New York) Water Agency and was dispensed via an automated stainless steel watering system. The diet was analyzed by the manufacturer for concentrations of specified heavy metals, hydrocarbons, organic phosphates and specified nutrients. Drinking water was analyzed by the Westchester County Public Health Laboratory (Valhalla, NY) for contaminants and specified microbes. The results of these analyses were negative and are on file at the AHF.

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TEXT TABLE 1
GROUP ASSIGNMENTS AND ANIMAL NUMBERS
29-DAY TERMINATION

Group 1, Control Males

1-1
1-2
2-2
3-2
5-1
5-2
7-1
7-2

Group 2, Control Females

8-1
10-1
11-1
11-2
12-1
12-2
13-1
14-1

Group 3, 120 ppm Males

15-1
15-2
16-1
16-2
17-1
17-2
18-1
18-2

Group 4, 120 ppm Females

19-1
19-2
20-1
20-2
21-1
21-2
22-1
22-2

Group 5, 470 ppm Males

23-1
23-2
24-1
24-2
25-1
25-2
26-1
26-2

Group 6, 470 ppm Females

27-1
27-2
28-1
28-2
29-1
29-2
30-1
30-2

Group 7, 1900 ppm Males

31-2
33-1
33-2
34-2
35-2
36-1
36-2
37-2

Group 8, 1900 ppm Females

39-2
41-1
41-2
42-2
43-1
43-2
44-1
44-2

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TEXT TABLE 2
GROUP ASSIGNMENTS AND ANIMAL NUMBERS
43-DAY TERMINATION

Group 1, Control Males

2-1
3-1
4-1
4-2
6-1
6-2

Group 2, Control Females

8-2
9-1
9-2
10-2
13-2
14-2

Group 7, 1900 ppm Males

31-1
32-1
32-2
34-1
35-1
37-1

Group 8, 1900 ppm Females

38-1
38-2
39-1
40-1
40-2
42-1

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TEST SUBSTANCE

Wingstay ®SN-1 (SN-1), a 1:11 (3, 6, 9-trioxaudecyl)-bis-(dodecylthio) propionate product (CAS 64253-30-1, lot number 130893, batch 7, and average molecular weight 684-706), is a white crystalline semisolid substance. It was mixed in powdered NIH-07 diet. One container of the final SN-1 was received from The Goodyear Tire & Rubber Company, Research Division, Akron, OH.

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METHODS

Compound Preparation and Administration

SN-1, dissolved in olive oil, was mixed in powdered NIH-07 diet. The concentration of olive oil (lot 6734; Dyets, Bethlehem, PA) in the diet was held constant at 2% for all groups. The diet mixtures were stored refrigerated in a closed container, protected from light, until used. The Hobart-mixers were used for the mixing of diets as per AHF SOP-429.

The test substance was given to rats for 28 days, with one concentration used for both genders of each dose group. In addition, six animals/gender from the control and high dose groups remained exposure-free until day 43. In the low dose groups (3 and 4) the concentration was 0.012% (120 ppm), set to deliver 7.5 mg/kg bw/day. In the mid dose groups (5 and 6) it was 0.047% (470 ppm), and in the high dose groups (7 and 8) it was 0.19% (1900 ppm), set to deliver 30 and 120 mg/kg bw/day, respectively.

For dose verification and stability analysis, the feed was poured into a bucket after mixing. Duplicate samples from the lowest and highest concentration diets were taken from the top and were stored at -20°C prior to analysis. For ascertainment of homogeneity, the feed was also poured into a bucket after mixing, as it was for dose verification. Duplicate samples from the top, middle and bottom for the lowest and highest dose levels of SN-1 were taken. For homogeneity, stability and dose verification, samples were taken and stored at -20°C. Approximately five grams or more of diet samples were collected from each batch prepared. All samples collected were not analyzed. Analysis is to be conducted at a later date pending development of a specific method of analysis by the Sponsor.

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Clinical Observations, Body Weights and Food Consumption

The animals were observed daily for mortality, morbidity and other cage-side observations as per AHF SOP-162 and SOP-182A.

Individual body weights were recorded on day -7 (for grouping), the first day of dosing, days 3 and 7, weekly thereafter during life, and at termination as per AHF SOP-155A.

Food consumption was calculated as daily intake and recorded from -5 to -1 days and over 5-day periods weekly for the duration of the study as per AHF SOP-160.

Hematology

For hematology, whole blood was collected via cardiac puncture under CO₂ anesthesia prior to necropsy and following overnight fasting. The following parameters were measured: RBC, hemoglobin, hematocrit, reticulocyte count, MCV, MCH, MCHC, RDW, WBC (see Glossary page 37), differential leukocyte count, platelet count, prothrombin time (PT) and activated partial thromboplastin time (APTT). Hematology was performed at "AniLytics, Inc.," of Gaithersburg, MD, under the direct supervision of Walter F. Loeb, V.M.D., Ph.D.

Clinical Chemistry

For clinical chemistry, blood was collected via cardiac puncture under CO₂ anesthesia prior to necropsy and following overnight fasting. Serum was obtained, and the following parameters were measured: Total protein, A/G ratio, glucose, triglycerides, total cholesterol, blood urea nitrogen, creatinine, albumin, globulin, sodium, potassium, chloride, calcium, inorganic phosphorus, iron, unsaturated (UIBC) and total iron binding capacity (TIBC), total bilirubin, serum glutamate-oxaloacetate

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transaminase (SGOT or aspartate aminotransferase-AST), serum glutamate puruvate transaminase (SGPT or alanine aminotransferase-ALT), alkaline phosphatase (ALP), transferrin, erythropoietin, and leucine aminopeptidase (LAP). Clinical chemistry was also performed at AniLytics.

Achieved Blood Levels of Test Substance

For serum test substance level monitoring, 0.5 ml serum/rat was obtained from three rats/group/gender during scheduled terminations and stored frozen (-20°C). Heparin was used as the anticoagulant. The tubes were centrifuged with the plasma drawn off prior to storage. The tubes were stored at -20°C for possible future SN-1 analysis.

Urinalysis

For urinalysis, three rats/gender/group were placed overnight in individual metabolism cages for urine collection during fasting prior to scheduled termination. The following parameters were analyzed: Urine appearance, volume, protein, glucose, specific gravity, occult blood, bilirubin, urobilinogen, pH and ketone bodies. Urinalysis was also performed at AniLytics.

Postmortem Examination

A. Necropsy and routine histopathology

Necropsies of randomly selected animals were performed on the following days: days 29 and 30, and 43. Euthanasia was achieved by CO₂ anesthesia with cardiac exsanguination. All animals were subjected to a complete gross postmortem examination (AHF SOP-050A). The liver, kidneys (pair), pituitary, uterus, heart, brain, spleen, thyroids, adrenals (pair), testes (pair with epididymides) and ovaries (pair) were weighed (AHF SOP-036A). Pituitary and thyroids (with parathyroids) were

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fixed prior to weighing. Organ weight to body weight percentages were calculated. The urinary bladder was distended with 10% neutral buffered formalin (NBF). The following tissues from each animal were preserved in NBF for histologic examination: gross lesions, brain, pituitary, thyroids/parathyroids, thymus, kidneys, lung, trachea, heart with aorta, tail bone, salivary glands (mandibular), mammary glands with skin, thigh muscle with sciatic nerve, eyes with optic nerve and Harderian gland, uterus, adrenals, liver, spleen, pancreas, testes with epididymides, ovaries, vagina, esophagus, stomach, duodenum, jejunum, ileum, colon, urinary bladder and mesenteric lymph node. The testes and epididymides were preserved in Bouin's solution. Eyes with optic nerve and Harderian gland were preserved in 6% Sorenson buffered (pH 7.2) glutaraldehyde solution for 24 hours, then trimmed and processed or placed in NBF until processing. The carcasses were discarded.

After fixation in NBF for 24 hours or more, tissue samples from each of three liver lobes (median right, median left and left lobes), kidneys, urinary bladder and any gross lesions present were trimmed, processed, embedded in paraffin, stained with H&E and evaluated microscopically. In addition, histopathology (H&E) was also performed on all tissues sampled in NBF (see above) from all control and high-dose animals. Treatment-related lesions observed in the high dose rats were examined in the mid and low dose animals.

B. Cell Proliferation Assessment

In order to monitor cell proliferation, separate liver and urinary bladder sections fixed in 10% NBF, were cut and stained for proliferating cell nuclear antigen (PCNA) according to AHF SOP-075A and SOP-076 (Galand 1989). Quantitation of PCNA-positive nuclei in the immunostained

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sections was performed on selected tissues from both 29- and 43-day terminations in the following manner: A square graticule (Olympus Instruments, Inc.) with 25 equal subdivisions was used for quantitating labeled cells at 200X magnification for liver, and at 400X magnification for urinary bladder. Each side of the graticule was confirmed by measurement with a second micrometer (Graticules, Ltd., Tonbridge, UK) as 0.125 mm, providing a square counting area that usually contains between 50 and 100 hepatocytes in the liver. All PCNA-labeled nuclei in this graticule area were counted. Counts were obtained for each animal from 25 graticule squares taken along the length of 1 to 3 liver lobe sections on the single glass slide. From the 25 graticule areas enumerated for each animal, between 1300 and 1800 nuclei per liver slide were counted. From the urinary bladder, counts were obtained for each animal from 20 longitudinal consecutive graticule squares having placed the bottom side of the graticule along the base of the urothelium of 1 to 2 urinary bladder sections on the single glass slide. From the 20 graticule areas enumerated for each animal, between 100 to 400 nuclei per urinary bladder slide were counted. Based on the above, the labeling indices for the liver and urinary bladder of each animal were calculated, indicating the percentage of positive nuclei out of the total counted number of hepatocellular or urothelial cell nuclei. The results were subjected to appropriate statistical analysis.

Statistical Analysis

Statistical analyses were performed on during-life body weights, final body weights, food consumption, absolute and relative organ weights, hematology, clinical chemistry, urinalysis and PCNA data. The analyses utilized ANOVA followed by Dunnett's test for comparison of several

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treatment groups versus the control (Dunnett, 1955) for all 29-day termination data and for all during-life data collected prior to termination. Since only two groups remained for each gender, Student's t-test was used to test each treatment group against its control for all during-life data and for all 43-day termination data.

Specimen, Raw Data and Final Report Storage

The protocol, all its and deviations, as well as all original data, tissue specimens, blood smears, blocks, slides, all raw data in study books (or copies thereof) and the final report are retained by the AHF for three years after completion of the final report. At the end of three years, the Sponsor will be contacted to discuss further storage arrangements for all study materials. Any remaining test substance is also returned to the Sponsor.

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RESULTS

Analytical Chemistry

Analytical data on stability, homogeneity and dose verification is to be conducted at a later date, based on development of specific method of analysis by the Sponsor.

General and Clinical Observations

No significant deviations occurred in either ambient temperature, relative humidity or any other variable in the animal room that would affect animal health or integrity of the study.

No compound-related clinical finding occurred in this study. All animals survived dosing in good health.

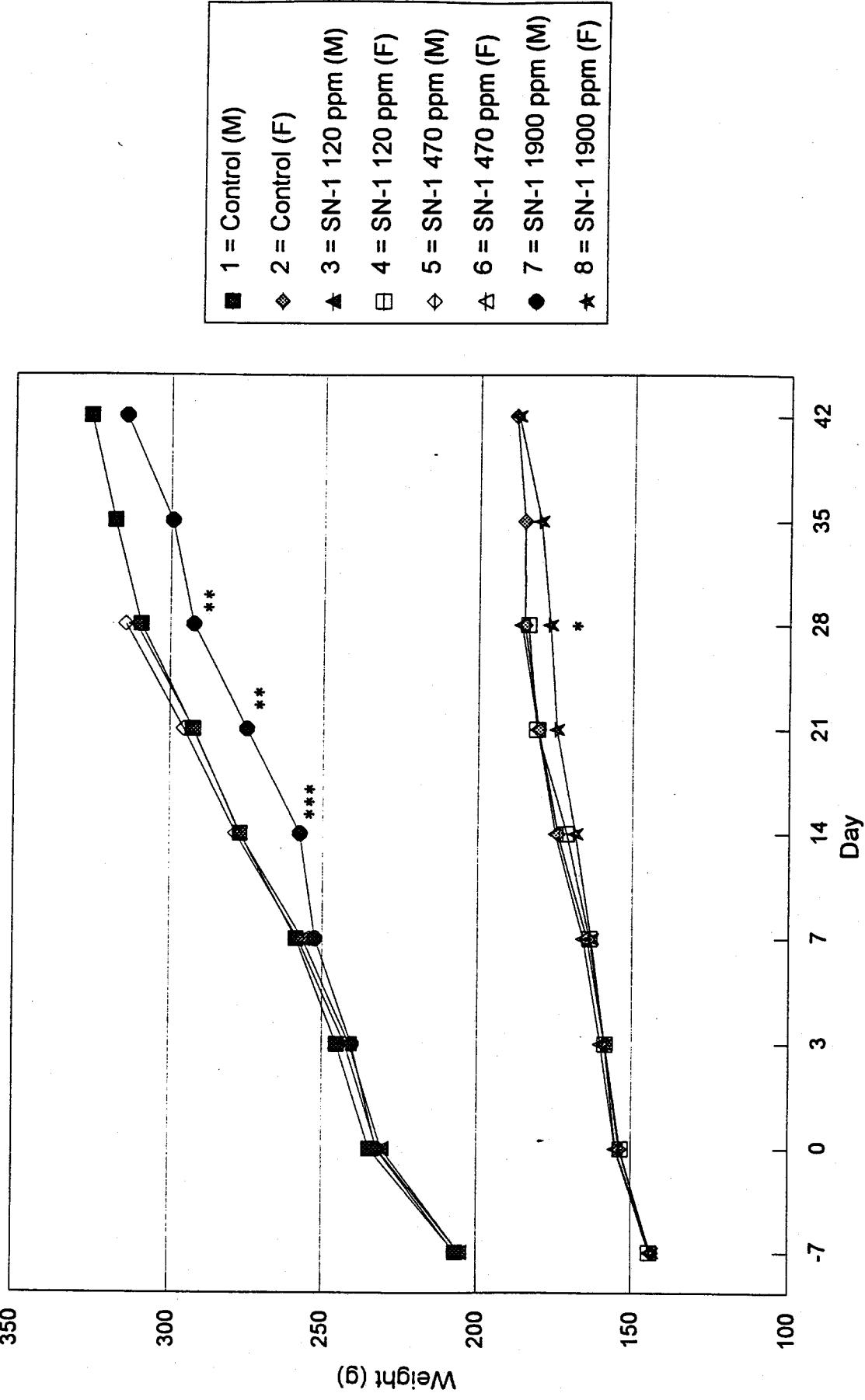
Body Weights and Food Consumption

The mean body weight gain data and pattern are given in Text Graph 1. The summary of during-life mean body weights is given in Table 1. The summary of mean body weights at scheduled terminations is given in Table 6. The individual during-life body weight data are given in Appendix 1. The individual body weights at scheduled terminations are presented in Appendices 9 and 10 for the 29-day and 43-day terminations, respectively.

Starting with day 7, body weight gain was reduced in animals of the high (1900 ppm) dose group in both genders. During the exposure-free period, there was a recovery trend in males and complete recovery in females. At the 29-day termination, in high dose males, the reduction was statistically significant at $p<0.05$ (7.2% reduction). The mean food consumption data pattern is given

TEXT GRAPH 1
Mean Body Weights - Males and Females

350



* significantly different from Control at $p<0.05$; ** $p<0.01$; *** $p<0.001$. The experimental means were compared to the Control by ANOVA followed by Dunnett's test at each time point from day -7 to day 28, after which Student's t-test was used.

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in Text Graph 2. The mean food consumption data are given in Table 2. The individual food consumption values are given in Appendix 2.

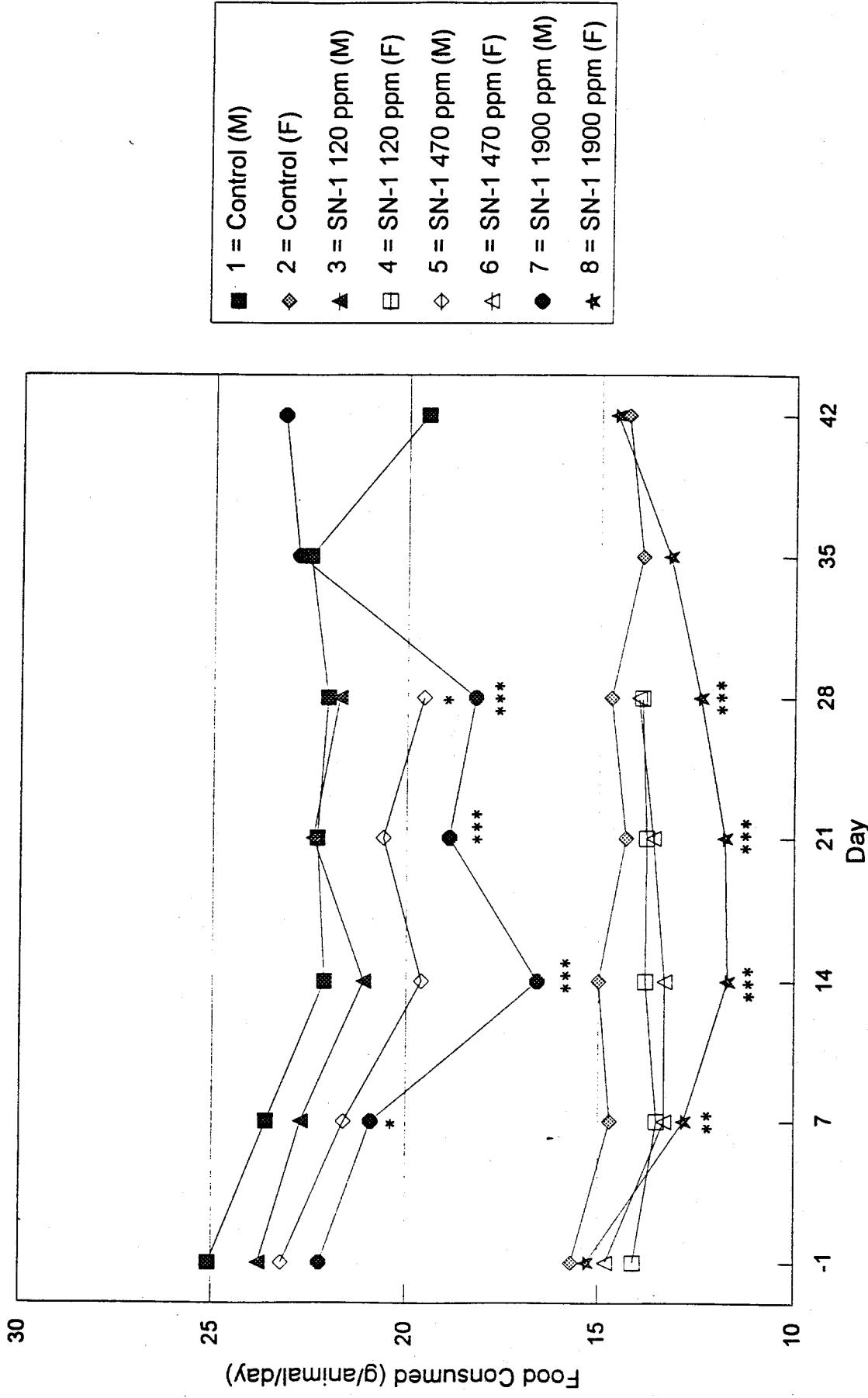
In both genders, food consumption was reduced in an exposure-related pattern after the first day of exposure and throughout exposure, reaching a nadir around day 14. During the 2-week exposure-free interval, food consumption recovered in both genders.

Hematology

A summary of all pertinent and compound-related hematology data is given in Table 3. The individual hematology data are given in Appendices 3 and 4 for the 29-day and 43-day terminations, respectively.

In both genders, at 29 days, the significant changes were mainly in high (1900 ppm) dose animals, and consisted of increases in mean corpuscular hemoglobin (MCH, males, $p<0.01$) and increases in mean corpuscular hemoglobin concentration (MCHC; in males, $p<0.001$). The MCH change persisted throughout the 2-week exposure-free recovery segment. In both genders, there was prolongation of PT (in males $p<0.05$, in females $p<0.001$) and reduction of APTT (in males not significant, in females $p<0.001$). In females, these two values were reduced also in the mid dose ($p<0.05$). In addition, during recovery, there were significant increases in hemoglobin (males, $p<0.01$), hematocrit (males, $p<0.01$), MCH (males, $p<0.05$), MCV (males, $p<0.01$; in females the value was decreased), and RDW (males, $p<0.05$). There were also reductions in PT (in males not significant, in females $p<0.05$) and decreases in APTT (in males not significant, in females $p<0.05$), indicating recovery for PT and no recovery for APTT. During recovery, there was eosinophilia

TEXT GRAPH 2
Mean Food Consumption - Males and Females



* significantly different from Control at $p<0.05$; ** $p<0.01$; *** $p<0.001$. The experimental means were compared to the Control by ANOVA followed by Dunnett's test at each time point from day -1 to day 28, after which Student's t-test was used.

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($p<0.001$) in high exposure females only, because the value in concurrent controls was zero. This is a coincidental finding and not compound-related.

Clinical Chemistry

A summary of all pertinent and compound-related clinical chemistry data is given in Table 4. The individual data are given in Appendices 5 and 6 for the 29-day and 43-day terminations, respectively.

In both genders, at 29 days, cholesterol and iron were significantly ($p<0.001$) decreased, while blood urea nitrogen (BUN) was significantly ($p<0.001$) increased, in high dose rats. These changes by day 43 did not recover. Moreover, in both genders, the BUN and iron level of significance remained high (at least $p<0.01$) during recovery. These three parameters were also changed in both genders in the mid-dose group. Furthermore, in males the iron was decreased in the low dose group as well. In addition, there were changes in the following parameters: Total proteins (males), triglycerides (males), LAP (females), TIBC (males), UIBC (females), transferrin (males), blood chlorides (males), blood phosphorus (both genders), calcium (males), alkaline phosphatase (females) and blood glucose (both genders).

Urinalysis

A summary of all pertinent and compound-related urinalysis data is given in Table 5. The individual data are given in Appendices 7 and 8, for the 29-day and 43-day terminations, respectively.

In both genders of the high dose group, at 29 days, there were ketone bodies in the urine ($p<0.01$), and lowering of the urine pH ($p<0.05$ in females of the high and mid dose groups). During

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recovery, the presence of ketone bodies persisted ($p<0.01$ in males), but the urinary pH values improved.

In mid exposure males, there was also a coincidental decrease ($p<0.05$) in specific gravity.

Postmortem Examination

A. Terminal body weights

The mean terminal body weights are given in Table 6. The individual data are given in Appendix 9 for the 29-day, and in Appendix 10 for the 43-day terminations.

In both genders, body weights in animals of the high dose group were decreased, albeit significantly ($p<0.05$) only in males. This decrease showed a recovery trend by day 43.

B. Organ Weights

The mean organ weights are given in Table 6. The individual absolute organ weight data are given in Appendices 11 and 12, for the 29-day and 43-day terminations, respectively. The individual relative organ weight data are given in Appendix 13 for the 29-day, and in Appendix 14 for the 43-day terminations.

Absolute liver weights were increased ($p<0.05$ in males and $p<0.001$ in females) at 29 days in animals of the high dose group. The thyroid weights of all SN-1 exposed groups were decreased ($p<0.001$ in males). These two weight changes were improved during recovery in males. On the other hand, a decrease in splenic weight was now evident in high dose males ($p<0.01$). Absolute ovarian weights were reduced ($p<0.01$) in high dose rats at 29 days. During the 2-week exposure-free interval, the ovarian weights improved. No other consistent or significant changes in absolute organ weight data were found either at 29 or 43 days.

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In males, of the high dose group, relative liver ($p<0.001$), kidney ($p<0.05$), heart ($p<0.01$), adrenal ($p<0.01$), brain ($p<0.01$) and testes ($p<0.01$) weights were increased at 29 days. The thyroid weights were decreased in all dose groups. In females of the high dose group, only the liver weights were increased ($p<0.001$), and the ovarian decreased. These changes improved during recovery, except for the liver and kidney weights. No other pertinent changes in relative organ weights were evident.

C. Gross findings

The summary of gross findings is given in Table 7 for the 29-day and 43-day terminations. The individual gross findings are given in Appendices 15 and 16 for the 29-day and 43-day terminations, respectively.

In both genders, at 29 and 43 days, no pertinent or consistent gross findings were present.

D. PCNA labeling indices

The summary of mean hepatocellular PCNA labeling indices is given in Table 8. The individual data are given in Appendices 17 and 18 for the 29-day and 43-day terminations, respectively.

The summary of mean urothelial PCNA labeling indices is given in Table 9. The individual data are given in Appendices 17 and 18 for the 29-day and 43-day terminations, respectively.

At 29 days, in both genders, the hepatocellular proliferation index of the high dose was increased ($p<0.001$). The increase was still evident (especially in females $p<0.001$) at 43 days. At 29 days, in both genders, the urothelial proliferation index (PI) in high dose animals was increased

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(males, $p<0.001$; females, $p<0.01$). In females, there was also an increase ($p<0.01$) in the mid dose group. At 43 days, the urothelial PI increase was still significant ($p<0.05$) in high dose females, whereas in males, the PI value was similar to controls.

E. Histopathological evaluation

The summary of histopathology findings is given in Table 10, for the 29-day and 43-day terminations. The individual data are given in Appendices 19 and 20 for the 29 and 43-day terminations, respectively.

Pertinent, consistent and compound-related microscopic findings were increased hepatocellular degeneration and apoptosis, cardiomyopathy with myocytic degeneration and fibrosis, and extensive congestion in the spleen. The hepatocellular and myocytic changes were more severe in males, whereas the splenic congestion was equally severe in both genders. By day 43, in both genders, there was improvement in the liver (not in females) and heart changes but not in the spleen. All the other observed microscopic changes were not compound-related and are known to occur in rats of this age and strain.

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DISCUSSION

The purpose of this study was to assess the subchronic toxicity of SN-1 in male and female F344 rats. SN-1 was administered in the diet for 28 days, followed by a 14-day exposure-free period of recovery. All standard during-life or postmortem parameters were monitored including hematology, clinical chemistry and urinalysis. In addition, the proliferating fractions from two tissues, i.e. liver and urinary bladder, were also monitored by staining for proliferating cell nuclear antigen (PCNA). This information was intended to help define subchronic toxicity and establish the doses to be used in chronic studies.

In both genders, body weight gain and food consumption were reduced in high dose animals. Both recovered during the 2-week exposure-free period. In males and females, the body weight gain decrease did not completely recover during recovery. In males and females, the food consumption decrease involved also the mid and low doses. The high dose terminal body weight reduction was 7.2% compared to controls in the males, and 4.5% in females. At the end of the exposure-free period these decreases had recovered in females and improved in males. This body weight gain reduction was accompanied by hypoproteinemia, hypoalbuminemia, hypoglobulinemia, prolonged prothrombin time, shortened APTT, hypocholesterolemia, hypotriglyceremia, increased BUN, and increased ketone bodies in the urine. In addition, in all dose levels in males and in mid and high dose levels in females, there was reduction of serum iron followed by a TIBC decrease. Furthermore, there was reduction in RDW values, which was followed by increases during recovery. Moreover, there was

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an increase in serum chlorides, which are usually increased in the presence of dehydration (Jacobs et al, 1990). Blood glucose was reduced and was accompanied by elevation of ALP, LAP (only in females) and P (only in females) and also a decrease in serum calcium in males. LAP helps in differentiating between hepatic and osseous etiologies of elevated ALP, indicating here that the etiology was hepatic (Jacobs et al, 1990). In urine, there is a decrease in pH, which was significant in mid and high dose levels in females.

In high dose males, significant increases occurred in relative liver, kidney, heart, brain, testicular and adrenal gland weights, and a decrease occurred in thyroid weight. In high dose females, increases occurred in relative liver weights and decreases occurred in ovarian weights. There was an increase in the proliferation indices of hepatocytes and urothelial cells in the high dose animals of both genders. In the hepatocytes, there was evidence of degeneration and apoptosis. In the females of the mid dose group, the urothelial PI was also increased, indicating that where the urine pH was reduced, the urothelial PI was increased. This is consistent with data in the literature (Iatropoulos et al, 1994). In addition, microscopically, there was evidence of cardiomyopathy. This, in all probability, was due to cardiac overload.

Based on all the significant data described above, it is apparent that SN-1 caused reduction in intracellular glucose availability and utilization, protein catabolism and interference with iron homeostasis resulting in hyposideremia. Thus, hypoglycemia and ketonuria (both more severe in males), which occur in chronic fasting and malnutrition, as well as hypcholesterolemia and hypotriglyceridemia, indicate that hepatic and extrahepatic tissues can not utilize glucose as a substrate

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for energy, catabolizing fat instead and inducing chronic hypoxia (Zilva and Pannall, 1975; Jacobs et al, 1990). In the liver, chronic hypoxia, hepatocellular degeneration and increase in apoptosis lead to increased hepatocellular proliferation (Iatropoulos and Williams, 1996). The ketonuria is associated with low urine pH (more severe in females), which in the absence of renal failure, as in the present study (Zilva and Pannall, 1975; Jacobs et al, 1990), contributes to the induction of urothelial proliferation (Iatropoulos et al, 1994). The urothelial proliferation is more severe in females and is also present in the mid dose, where low pH is evident. Furthermore, the hypoproteinemia and the improper utilization of glucose in hepatocytes (more severe in males) are associated with high BUN values, which are present in both higher doses of both genders.

All of these findings occurred in the presence of proper renal functioning, albeit with indication of cardio-renal and circulatory overload reflected in kidney, heart and adrenal weight increases. Moreover, protein catabolism in the liver impacts also on the homeostasis of many substances, such as estrogen, hemosiderin, ceruloplasmin, thromboplastin and prothrombin (Jacobs et al, 1990; DeMott, 1990; Tilzer and DeMott, 1990; Crawford et al, 1992; Ojeda, 1992). Furthermore, protein catabolism, reduced renal blood flow and increased BUN reflect dehydration and hypovolemia (Jacobs et al, 1990). These two conditions are also associated with increases in serum chlorides and brain weight (more severe in males) (Jacobs et al, 1990). The hyposideremia, which is accompanied by increased MCHC and reduced RDW, indicates inadequate absorption of iron and conditions of chronic fasting with the RBC assuming more compact spherocyte-like appearance (Busby, 1970; Jain, 1986; Tilzer and DeMott, 1990). On the other hand, the decreases

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in TIBC and transferrin reflect hypoproteinemia (Tilzer and DeMott, 1990). Finally, the increases in alkaline phosphatase in females are due to hepatocellular and not osseous changes as confirmed by concomitant LAP increases (Jacobs et al, 1990).

In conclusion, under the conditions of this study, SN-1 at 1900 ppm in both genders caused a reduction in intracellular glucose availability and utilization, protein catabolism, as well as inadequate absorption of iron. These lead to ketonuria, hypoproteinemia, hypolipidemia, hyposideremia, increased BUN, dehydration, hypovolemia and hypoxia in both hepatic and extrahepatic tissues. Moreover, these changes result in overload of the cardio-renal-vascular system without evidence of renal failure and increases in hepatocellular and urothelial cell proliferation. During recovery, there is improvement, but not complete reversal of these findings.

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GLOSSARY
FOR TABLES AND APPENDICES

A/G RATIO	=	Albumin/globulin ratio	MCHC	=	Mean corpuscular hemoglobin concentration
ALK PHOS	=	Alkaline phosphatase (total)	MCV	=	Mean corpuscular volume
ALT-SGPT	=	Alanine aminotransferase	METAS	=	Metamyelocytes
APTT	=	Activated partial thromboplastin time	MONOS	=	Monocytes
AST-SGOT	=	Aspartate aminotransferase	MYELOS	=	Mycelocytes
ATLYMPH	=	Atypical lymphocyte	PHOS	=	Phosphorus
BANDS	=	Band cells (young neutrophils with nonsegmented nucleus)	PLTS	=	Platelets
BASOS	=	Basophils	PROTIME	=	Prothrombin time
BLOOD	=	Occult blood	RBC	=	Red blood cell count (erythrocyte count)
BUN	=	Blood urea nitrogen	RDW	=	Red cell distribution width
CHOLEST	=	Cholesterol (total)	RETIC	=	Reticulocyte count
CREAT	=	Creatinine	SEGS	=	Mature (segmented) polymorphonuclear neutrophil
EOSN	=	Eosinophil	T BILI	=	Bilirubin (total)
EPO	=	Erythropoietin	T-FERRIN	=	Transferrin
GLUCO	=	Glucose	TIBC	=	Total iron binding capacity
HCT	=	Hematocrit	TP	=	Total protein
HGB	=	Hemoglobin	TRIG	=	Triglycerides
LAP	=	Leucine aminopeptidase	UBC	=	Unsaturated iron binding capacity
LYMPHS	=	Lymphocytes	UROBIL	=	Urobilinogen
MCH	=	Mean corpuscular hemoglobin	UR. SP.GR.	=	Urine specific gravity
			WBC	=	White blood cell count

Note on Numerical Data in Tables and Appendices:

In the tables and appendices, the values shown are, in some cases, rounded off to a fixed number of significant digits, with a higher number of digits than tabulated being used for calculation of means and standard deviations. Therefore, recalculation of means and standard deviations from the tabulated data may, in some instances, lead to minor and inconsequential variations, particularly in the last significant digit.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
 SUMMARY OF MEAN BODY WEIGHT DATA (g)

		DAY								
		-7	0	3	7	14	21	28	35	42
Group and Treatment	n	M	SD	M	SD	M	SD	M	SD	M
1 Control - No Treatment Males	n M SD	14 206.7 7.00	14 234.4 8.78	14 245.2 8.28	14 258.7 9.40	14 277.1 7.91	14 292.6 8.22	14 309.4 9.88	14 318.0 9.50	6 325.8 28.27
3 SN-1 120 ppm Males	n M SD	8 205.4 7.65	8 230.5 7.96	8 241.0 9.09	8 256.5 10.23	8 277.3 13.32	8 292.5 14.66	8 311.1 15.74	8 --- ---	---
5 SN-1 470 ppm Males	n M SD	8 205.4 7.60	8 231.8 11.73	8 242.4 12.22	8 257.9 14.88	8 278.5 15.05	8 295.6 16.79	8 314.3 18.56	8 --- ---	---
7 SN-1 1900 ppm Males	n M SD	14 206.9 6.68	14 232.1 10.52	14 240.3 9.37	14 252.4 8.19	14 257.4*** 12.11	14 275.0** 10.68	14 292.4** 12.57	6 299.5 15.58	6 314.3 13.52

n = Number of Animals

M = Mean

SD = Standard Deviation

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

For days -7 to 28, means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

For days 35 and 42, the experimental mean was compared to the Control mean using Student's t-test.

TABLE 1 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF MEAN BODY WEIGHT DATA (g)

Group and Treatment		DAY									
		-7	0	3	7	14	21	28	35	42	
2	n	14	14	14	14	14	14	14	6	6	
Control - No Treatment Females	M	143.5	153.8	158.6	164.4	173.9	180.3	185.1	185.3	188.2	
	SD	4.29	4.90	5.15	5.26	5.82	6.76	7.03	9.09	8.77	
4	n	8	8	8	8	8	8	8	--	--	
SN-1 120 ppm Females	M	144.0	153.4	158.4	163.5	171.0	181.0	183.9	--	--	
	SD	5.90	5.76	5.07	5.42	5.66	6.76	5.41	--	--	
6	n	8	8	8	8	8	8	8	--	--	
SN-1 470 ppm Females	M	144.0	155.1	159.9	165.6	174.8	180.6	186.1	--	--	
	SD	6.09	7.30	7.94	6.93	8.19	7.46	10.63	--	--	
8	n	14	14	14	14	14	14	14	6	6	
SN-1 1900 ppm Females	M	143.4	154.8	158.6	163.0	168.3	174.4	176.7*	180.0	187.5	
	SD	4.96	4.98	5.81	5.87	7.55	6.33	8.86	8.15	8.50	

n = Number of Animals

M = Mean

SD = Standard Deviation

* significantly different from Control at p<0.05

For days -7 to 28, means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

For days 35 and 42, the experimental mean was compared to the Control mean using Student's t-test.

TABLE 2
FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
SUMMARY OF FOOD CONSUMPTION DATA (g/animal/day)

Group and Treatment	n	M	DAY					
			-1	7	14	21	28	35
1 Control - No Treatment Males	n M	14 25.1	14 23.6	14 22.1	14 22.3	14 22.0	14 22.5	6 19.5
3 SN-1 120 ppm Males	n M	8 23.8	8 22.7	8 21.1	8 22.4	8 21.7	-- --	-- --
5 SN-1 470 ppm Males	n M	8 23.2	8 21.6	8 19.6	8 20.6	8 19.6*	-- --	-- --
7 SN-1 1900 ppm Males	n M	14 22.2	14 20.9*	14 16.6***	14 18.9***	14 18.2***	6 22.8	6 23.2

n = Number of Animals
M = Mean

* significantly different from Control at p<0.05

*** significantly different from Control at p<0.001

For days -1 to 28, means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

For days 35 and 42, the experimental mean was compared to the Control mean using Student's t-test.

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TABLE 2 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF FOOD CONSUMPTION DATA (g/animal/day)

Group and Treatment		DAY					
		-1	7	14	21	28	35
2	n M	14 15.7	14 14.7	14 15.0	14 14.3	14 14.7	6 13.9
Control - No Treatment Females							6 14.3
4	n M	8 14.1	8 13.5	8 13.8	8 13.8	8 13.9	---
SN-1 120 ppm Females							---
6	n M	8 14.8	8 13.3	8 13.3	8 13.6	8 14.0	---
SN-1 470 ppm Females							---
8	n M	14 15.3	14 12.8**	14 11.7***	14 11.8***	14 12.4***	6 13.2
SN-1 1900 ppm Females							6 14.6

n = Number of Animals

M = Mean

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

For days -1 to 28, means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

For days 35 and 42, the experimental mean was compared to the Control mean using Student's t-test.

TABLE 3
FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
SUMMARY OF COMPOUND-RELATED HEMATOLOGY DATA

		29-DAY SACRIFICE (MALES)											
GROUP IDENTIFICATION		RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLTS	MONOS	EOSN	PROTIME	APTT
1. Control Diet	Mean	7.863	15.82	46.88	59.62	20.12	33.73	26.68	609.7	0.078	0.008	15.03	36.75
	SD	0.170	0.50	1.73	1.35	0.54	0.55	0.25	45.4	0.048	0.020	1.42	7.70
3. SN-1 120 ppm	Mean	7.406*	15.42	44.86	60.64	20.84	34.38	26.66	673.8	0.032	0.042	15.47	30.98
	SD	0.298	0.45	0.82	1.55	0.68	0.64	0.62	31.7	0.046	0.066	0.91	9.05
5. SN-1 470 ppm	Mean	7.950	16.37	47.52	59.75	20.58	34.45	26.22	737.7***	0.050	0.103	15.52	37.10
	SD	0.175	0.20	1.50	0.87	0.39	0.97	0.75	43.9	0.063	0.145	2.07	7.55
7. SN-1 1900 ppm	Mean	7.557	16.05	44.85	59.35	21.25**	35.82***	26.03	821.2***	0.035	0.053	18.08*	25.76
	SD	0.301	0.43	2.05	0.58	0.46	0.95	1.04	71.7	0.042	0.067	0.54	5.19

RBC = Red blood count (millions/microliter)

HGB = Hemoglobin (grams/deciliter)

HCT = Hematocrit (%)

MCV = Mean corpuscular volume (femtoliters)

MCH = Mean corpuscular hemoglobin (picograms)

MCHC = Mean corpuscular hemoglobin concentration (%)

RDW = Red cell distribution width (%)

PLTS = Platelets (thousands/microliter)

MONOS = Monocytes (thousands/microliter)

EOSN = Eosinophils (thousands/microliter)

PROTIME = Prothrombin time (seconds)

APTT = Activated partial thromboplastin time (seconds)

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

TABLE 3 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
SUMMARY OF COMPOUND-RELATED HEMATOLOGY DATA

29-DAY SACRIFICE (FEMALES)													
GROUP IDENTIFICATION	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLTS	MONOS	EOSN	PROTIME	APTT	
2. Control Diet	Mean SD	7.350 0.331	16.05 0.67	45.65 1.77	62.10 0.62	21.83 0.30	35.15 0.60	22.13 2.51	704.3 138.3	0.072 0.051	0.062 0.077	12.60 2.78	40.80 4.09
4. SN-1 120 ppm	Mean SD	7.117 0.314	15.67 0.16	44.02 1.41	61.87 0.90	22.07 0.86	35.63 1.02	20.18 3.80	770.3 597	0.038 0.034	0.025 0.032	14.30 3.71	35.62 8.53
6. SN-1 470 ppm	Mean SD	7.278 0.265	15.95 0.60	44.75 1.93	61.50 0.46	21.93 0.59	35.65 1.03	17.97 3.38	781.3 44.3	0.070 0.025	0.048 0.051	16.38* 1.58	28.87* 7.29
8. SN-1 1900 ppm	Mean SD	7.262 0.310	15.82 0.49	43.93 1.95	60.50** 0.54	21.80 0.43	36.03 0.92	17.97 3.17	783.5 103.5	0.040 0.022	0.008 0.020	19.05*** 0.23	22.72*** 4.32

RBC = Red blood count (millions/microliter)

HGB = Hemoglobin (grams/deciliter)

HCT = Hematocrit (%)

MCV = Mean corpuscular volume (femtoliters)

MCH = Mean corpuscular hemoglobin (picograms)

MCHC = Mean corpuscular hemoglobin concentration (%)

RDW = Red cell distribution width (%)

PLTS = Platelets (thousands/microliter)

MONOS = Monocytes (thousands/microliter)

EOSN = Eosinophils (thousands/microliter)

PROTIME = Prothrombin time (seconds)

APTT = Activated partial thromboplastin time (seconds)

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

TABLE 3 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
 SUMMARY OF COMPOUND-RELATED HEMATOLOGY DATA

		43-DAY SACRIFICE (MALES)											
GROUP IDENTIFICATION		RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLTS	MONOS	EOSN	PROTIME	APTT
1. Control Diet	Mean	7.942	15.57	44.92	56.57	19.58	34.65	21.42	823.0	0.037	0.078	16.03	33.03
	SD	0.193	0.41	0.86	0.39	0.23	0.45	2.48	193.5	0.031	0.081	0.91	2.83
7. SN-1 1900 ppm	Mean	8.242	16.57**	47.63**	57.80**	20.12*	34.78	24.35*	800.5	0.090*	0.070	15.47	31.23
	SD	0.289	0.41	1.21	0.60	0.39	0.64	1.57	52.4	0.034	0.065	0.61	3.86

RBC = Red blood count (millions/microliter)

HGB = Hemoglobin (grams/decliliter)

HCT = Hematocrit (%)

MCV = Mean corpuscular volume (femtoliters)

MCH = Mean corpuscular hemoglobin (picograms)

MCHC = Mean corpuscular hemoglobin concentration (%)

RDW = Red cell distribution width (%)

PLTS = Platelets (thousands/microliter)

MONOS = Monocytes (thousands/microliter)

EOSN = Eosinophils (thousands/microliter)

PROTIME = Prothrombin time (seconds)

APTT = Activated partial thromboplastin time (seconds)

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

Experimental means between the groups were compared with the Control using Student's t-test.

TABLE 3 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
 SUMMARY OF COMPOUND-RELATED HEMATOLOGY DATA

43-DAY SACRIFICE (FEMALES)									
GROUP IDENTIFICATION	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLTS	MONOS EOSN PROTOME APTT
2. Control Diet	Mean 7.583	15.68	46.38	61.17	20.68	33.83	15.90	764.5	0.077 0.000 15.77 36.83
	SD 0.301	0.52	1.78	0.29	0.35	0.56	0.49	39.9	0.045 0.000 2.41 4.70

RBC = Red blood count (millions/microliter)

HGB = Hemoglobin (grams/deciliter)

HCT = Hematocrit (%)

MCV = Mean corpuscular volume (femtoliters)

MCH = Mean corpuscular hemoglobin (picograms)

MCHC = Mean corpuscular hemoglobin concentration (%)

RDW = Red cell distribution width (%)

PLTS = Platelets (thousands/microliter)

MONOS = Monocytes (thousands/microliter)

EOSN = Eosinophils (thousands/microliter)

PROTIME = Prothrombin time (seconds)

APTT = Activated partial thromboplastin time (seconds)

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

Experimental means between the groups were compared with the Control using Student's t-test.

TABLE 4
FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
SUMMARY OF COMPOUND-RELATED CLINICAL CHEMISTRY DATA

		29-DAY SACRIFICE (MALES)							
GROUP IDENTIFICATION	TOTAL PROTEIN	ALBUMIN	GLOBULIN	CHOLEST	TRIG	LAP	IRON	UIBC	TIBC
1. Control Diet	Mean SD	7.40 0.22	4.73 0.19	2.67 0.08	55.7 3.4	117.7 22.7	51.63 1.94	126.0 22.3	385.3 35.4
3. SN-1 120 ppm	Mean SD	7.18 0.13	4.57 0.10	2.62 0.12	52.7 5.0	79.8* 28.0	46.60 1.02	90.3* 16.9	397.0 20.9
5. SN-1 470 ppm	Mean SD	7.35 0.26	4.60 0.20	2.75 0.22	51.7 10.0	76.8* 21.8	44.12 12.22	52.8*** 25.5	487.3 21.7
7. SN-1 1900 ppm	Mean SD	6.85*** 0.23	4.47* 0.21	2.38* 0.23	32.0*** 2.5	68.8** 24.3	51.20 2.38	50.3*** 27.6	458.0** 35.4
									510.8 29.7

TOTAL PROTEIN = Total protein (grams/deciliter)

ALBUMIN = Albumin (grams/deciliter)

GLOBULIN = Globulin (grams/deciliter)

CHOLEST = Cholesterol (milligrams/deciliter)

TRIG = Triglyceride (milligrams/deciliter)

LAP = Leucine aminopeptidase (units/liter)

IRON = Serum iron (micrograms/deciliter)

TIBC = Total iron binding capacity (micrograms/deciliter)

UIBC = Unsaturated iron binding capacity (micrograms/deciliter)

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

TABLE 4 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
 SUMMARY OF COMPOUND-RELATED CLINICAL CHEMISTRY DATA

		29-DAY SACRIFICE (MALES)						
GROUP IDENTIFICATION	T-FERRIN	EPO	CL-	CALCIUM	PHOS	ALK PHOS	GLUCOSE	BUN
1. Control Diet	Mean SD	400.8 26.9	52.145 8.308	97.7 1.9	12.12 0.73	11.05 2.39	185.2 13.2	222.5 113.9
3. SN-1 120 ppm	Mean SD	375.0 19.7	55.550 15.455	99.5 0.5	11.65 0.39	10.58 1.10	182.7 9.1	134.0* 19.1
5. SN-1 470 ppm	Mean SD	361.7* 20.4	43.403 6.688	100.2* 1.9	11.62 0.57	11.53 1.49	202.0 15.9	156.0 23.9
7. SN-1 1900 ppm	Mean SD	359.2** 14.6	42.980 8.348	101.3*** 1.0	11.07** 0.23	10.70 0.28	203.5 16.4	139.5 9.4

T-FERRIN = Transferrin (milligrams/deciliter)

EPO = Erythropoietin (million units/milliliter)

CL- = Chloride (millequivalents/liter)

CALCIUM = Calcium, total (milligrams/deciliter)

PHOS = Phosphorus (milligrams/deciliter)

ALK PHOS = Alkaline phosphatase, total (units/liter)

GLUCOSE = Glucose (milligrams/deciliter)

BUN = Blood urea nitrogen (milligrams/deciliter)

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

TABLE 4 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
SUMMARY OF COMPOUND-RELATED CLINICAL CHEMISTRY DATA

		29-DAY SACRIFICE (FEMALES)							
GROUP IDENTIFICATION	TOTAL PROTEIN	ALBUMIN	GLOBULIN	CHOLEST	TRIG	LAP	IRON	UIBC	TIBC
2. Control Diet	Mean SD	6.97 0.22	4.75 0.29	2.22 0.20	79.5 9.4	65.8 10.3	50.23 3.04	261.0 85.2	180.8 84.3
4. SN-1 120 ppm	Mean SD	6.80 0.29	4.62 0.21	2.18 0.21	74.7 6.7	50.3 15.4	49.78 0.90	266.5 87.5	167.5 88.3
6. SN-1 470 ppm	Mean SD	6.70 0.44	4.52 0.24	2.18 0.22	62.5** 3.5	47.5 11.3	49.78 0.97	135.8** 37.7	265.0 58.2
8. SN-1 1900 ppm	Mean SD	6.57 0.26	4.58 0.23	1.98 0.13	48.5*** 7.8	79.2 18.5	54.18** 1.90	135.7** 29.9	287.7* 48.8
TOTAL PROTEIN = Total protein (grams/deciliter)									
ALBUMIN = Albumin (grams/deciliter)									
GLOBULIN = Globulin (grams/deciliter)									
CHOLEST = Cholesterol (milligrams/deciliter)									
TRIG = Triglyceride (milligrams/deciliter)									
LAP = Leucine aminopeptidase (units/liter)									
IRON = Serum iron (micrograms/deciliter)									
TIBC = Total iron binding capacity (micrograms/deciliter)									
UIBC = Unsaturated iron binding capacity (micrograms/deciliter)									

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

TABLE 4 (cont'd)
 FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
 SUMMARY OF COMPOUND-RELATED CLINICAL CHEMISTRY DATA

		29-DAY SACRIFICE (FEMALES)						
GROUP IDENTIFICATION	T-FERRIN	EPO	CL-	CALCIUM	PHOS	ALK PHOS	GLUCOSE	BUN
2. Control Diet	Mean SD	345.0 16.7	48.603 8.802	104.2 1.7	11.23 0.12	10.87 1.87	142.5 25.6	156.3 31.1
4. SN-1 120 ppm	Mean SD	349.2 12.4	45.730 5.654	104.0 2.4	11.02 0.31	9.88 0.89	155.5 10.9	122.5* 7.5
6. SN-1 470 ppm	Mean SD	335.0 20.7	43.637 7.811	106.2 3.0	11.08 0.38	10.65 0.57	164.5 18.9	140.0 7.5 2.1
8. SN-1 1900 ppm	Mean SD	342.5 9.9	43.542 5.039	106.5 3.1	11.15 0.67	11.68 1.80	196.8*** 25.5	129.7 5.4 16.7* 20.9 1.2 2.9

T-FERRIN = Transferrin (milligrams/deciliter)

EPO = Erythropoietin (milliunits/milliliter)

CL- = Chloride (millequivalents/liter)

CALCIUM = Calcium, total (milligrams/deciliter)

PHOS = Phosphorus (milligrams/deciliter)

ALK PHOS = Alkaline phosphatase, total (units/liter)

GLUCOSE = Glucose (milligrams/deciliter)

BUN = Blood urea nitrogen (milligrams/deciliter)

SD = Standard Deviation

* significantly different from Control at p<0.05

*** significantly different from Control at p<0.001

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

TABLE 4 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
 SUMMARY OF COMPOUND-RELATED CLINICAL CHEMISTRY DATA

		43-DAY SACRIFICE (MALES)							
GROUP IDENTIFICATION	TOTAL PROTEIN	ALBUMIN	GLOBULIN	CHOLEST	TRIG	LAP	IRON	UIBC	TIBC
1. Control Diet	Mean SD	7.37 0.28	4.78 0.21	2.58 0.12	67.3 12.8	127.5 27.8	47.60 5.75	110.8 22.1	391.0 42.3
7. SN-1 1900 ppm	Mean SD	7.05 0.28	4.68 0.15	2.37* 0.21	44.3** 10.7	83.8* 24.4	48.90 2.25	58.7*** 11.6	456.3* 20.1

TOTAL PROTEIN = Total protein (grams/deciliter)

ALBUMIN = Albumin (grams/deciliter)

GLOBULIN = Globulin (grams/deciliter)

CHOLEST = Cholesterol (milligrams/deciliter)

TRIG = Triglyceride (milligrams/deciliter)

LAP = Leucine aminopeptidase (units/liter)

IRON = Serum iron (micrograms/deciliter)

TIBC = Total iron binding capacity (micrograms/deciliter)

UIBC = Unsaturated iron binding capacity (micrograms/deciliter)

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

Experimental means between the groups were compared with the Control using Student's t-test.

TABLE 4 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
 SUMMARY OF COMPOUND-RELATED CLINICAL CHEMISTRY DATA

43-DAY SACRIFICE (MALES)							
GROUP IDENTIFICATION	T-FERRIN	EPO	CL-	CALCIUM	PHOS	ALK PHOS	GLUCOSE BUN
1. Control Diet	Mean SD	406.7 29.8	40.785 9.377	95.0 1.1	11.38 0.25	8.55 0.74	145.0 27.9
7. SN-1 1900 ppm	Mean SD	382.5 9.9	43.837 7.839	98.2* 2.6	11.37 0.32	9.68* 0.78	161.8 23.1

T-FERRIN = Transferrin (milligrams/deciliter)

EPO = Erythropoietin (million units/milliliter)

CL- = Chloride (millequivalents/liter)

CALCIUM = Calcium, total (milligrams/deciliter)

PHOS = Phosphorus (milligrams/deciliter)

ALK PHOS = Alkaline phosphatase, total (units/liter)

GLUCOSE = Glucose (milligrams/deciliter)

BUN = Blood urea nitrogen (milligrams/deciliter)

SD = Standard Deviation

* significantly different from Control at p<0.05

*** significantly different from Control at p<0.001

Experimental means between the groups were compared with the Control using Student's t-test.

TABLE 4 (cont'd)
 FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
 SUMMARY OF COMPOUND-RELATED CLINICAL CHEMISTRY DATA

		43-DAY SACRIFICE (FEMALES)							
GROUP IDENTIFICATION	TOTAL PROTEIN	ALBUMIN	GLOBULIN	CHOLEST	TRIG	LAP	IRON	UIBC	TIBC
2. Control Diet	Mean SD	7.25 0.52	4.85 0.32	2.40 0.21	82.0 11.8	61.2 17.1	54.85 1.72	266.3 72.4	180.3 85.3
8. SN-1 1900 ppm	Mean SD	6.77 0.41	4.58 0.15	2.18 0.32	64.8* 10.3	68.8 18.9	52.38 3.46	113.2** 22.7	319.2* 25.1

TOTAL PROTEIN = Total protein (grams/deciliter)

ALBUMIN = Albumin (grams/deciliter)

GLOBULIN = Globulin (grams/deciliter)

CHOLEST = Cholesterol (milligrams/deciliter)

TRIG = Triglyceride (milligrams/deciliter)

LAP = Leucine aminopeptidase (units/liter)

IRON = Serum iron (micrograms/deciliter)

TIBC = Total iron binding capacity (micrograms/deciliter)

UIBC = Unsaturated iron binding capacity (micrograms/deciliter)

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

Experimental means between the groups were compared with the Control using Student's t-test.

TABLE 4 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
 SUMMARY OF COMPOUND-RELATED CLINICAL CHEMISTRY DATA

43-DAY SACRIFICE (FEMALES)						
GROUP IDENTIFICATION	T-FERRIN	EPO	CL-	CALCIUM	PHOS	ALK PHOS
2. Control Diet	Mean SD	343.3 16.9	51.613 12.069	100.0 2.0	11.35 0.37	8.30 0.72
8. SN-1 1900 ppm	Mean SD	354.2 15.9	41.297 9.718	101.7 2.8	10.93 0.32	115.3 1.21
					10.23** 1.21	143.3 23.3
					157.7** 23.3	15.0 8.4
						22.2*** 8.4
						2.2 1.6

T-FERRIN = Transferrin (milligrams/deciliter)

EPO = Erythropoietin (milliunits/milliliter)

CL- = Chloride (millequivalents/liter)

CALCIUM = Calcium, total (milligrams/deciliter)

PHOS = Phosphorus (milligrams/deciliter)

ALK PHOS = Alkaline phosphatase, total (units/liter)

GLUCOSE = Glucose (milligrams/deciliter)

BUN = Blood urea nitrogen (milligrams/deciliter)

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

Experimental means between the groups were compared with the Control using Student's t-test.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF COMPOUND-RELATED URINALYSIS DATA

<u>29-DAY SACRIFICE (MALES)</u>			
GROUP IDENTIFICATION	SPECIFIC GRAVITY	pH	KETONES
1. Control Diet	Mean SD	1.0500 0.0020	6.67 0.29
3. SN-1 120 ppm	Mean SD	1.0427 0.0042	6.50 0.00
5. SN-1 470 ppm	Mean SD	1.0387* 0.0058	5.0 0.0
7. SN-1 1900 ppm	Mean SD	1.0433 0.0058	31.7** 14.4

SPECIFIC GRAVITY = Specific gravity (SG units)

pH = Measure of hydrogen ion activity

VOLUME = Volume (milliliters)

KETONES = Ketones (milligrams/deciliter)

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

TABLE 5 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF COMPOUND-RELATED URINALYSIS DATA

29-DAY SACRIFICE (FEMALES)			
GROUP IDENTIFICATION	SPECIFIC GRAVITY	pH	KETONES
2. Control Diet	Mean SD	1.0190 0.0061	6.67 0.29
4. SN-1 120 ppm	Mean SD	1.0287 0.0126	6.33 0.29
6. SN-1 470 ppm	Mean SD	1.0250 0.0017	6.00* 0.00
8. SN-1 1900 ppm	Mean SD	1.0300 0.0115	6.00* 0.00

SPECIFIC GRAVITY = Specific gravity (SG units)

pH = Measure of hydrogen ion activity

VOLUME = Volume (milliliters)

KETONES = Ketones (milligrams/deciliter)

SD = Standard Deviation

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

TABLE 5 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF COMPOUND-RELATED URINALYSIS DATA

43-DAY SACRIFICE (MALES)			
GROUP IDENTIFICATION	SPECIFIC GRAVITY	pH	KETONES
1. Control Diet	Mean SD	1.0253 0.0081	6.67 0.29
7. SN-1 1900 ppm	Mean SD	1.0380 0.0087	40.0** 0.0

SPECIFIC GRAVITY = Specific gravity (SG units)

pH = Measure of hydrogen ion activity

VOLUME = Volume (milliliters)

KETONES = Ketones (milligrams/deciliter)

SD = Standard Deviation

** significantly different from Control at p<0.01

Experimental means between the groups were compared with the Control using Student's t-test.

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TABLE 5 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF COMPOUND-RELATED URINALYSIS DATA

GROUP IDENTIFICATION	SPECIFIC GRAVITY	pH	KETONES	
			4.3-DAY SACRIFICE (FEMALES)	
2. Control Diet	Mean SD	1.0220 0.0106	6.33 0.29	0.0 0.0
8. SN-1 1900 ppm	Mean SD	1.0327 0.0064	6.00 0.00	6.7 7.6

SPECIFIC GRAVITY = Specific gravity (SG units)

pH = Measure of hydrogen ion activity

VOLUME = Volume (milliliters)

KETONES = Ketones (milligrams/deciliter)

SD = Standard Deviation

There was no significant difference from Control.

Experimental means between the groups were compared with the Control using Student's t-test.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF BODY AND ABSOLUTE ORGAN WEIGHT MEANS (g)

29-DAY SACRIFICE (MALES)				
GROUP # TREATMENT	1 Control - No Treatment	3 SN-1 120 ppm	5 SN-1 470 ppm	7 SN-1 1900 ppm
Body Weight				
Number of Animals	8	8	8	8
Mean	290.9	293.9	293.6	269.9*
Range	280-309	275-321	267-326	251-287
Standard Deviation	8.51	17.48	18.12	12.41
Liver Weight				
Number of Animals	8	8	8	8
Mean	8.681	8.703	9.102	9.832*
Range	8.171-9.249	7.810-10.600	7.990-10.627	8.876-10.927
Standard Deviation	0.464	0.929	0.885	0.696
Kidney Weight				
Number of Animals	8	8	8	8
Mean	2.131	2.128	2.093	2.121
Range	1.999-2.431	1.846-2.463	1.901-2.337	1.839-2.470
Standard Deviation	0.140	0.190	0.142	0.195
Pituitary Weight				
Number of Animals	8	8	8	8
Mean	0.0101	0.0095	0.0095	0.0096
Range	0.0084-0.0125	0.0085-0.0109	0.0085-0.0109	0.0078-0.0118
Standard Deviation	0.0015	0.0008	0.0008	0.0012
Thyroid Weight				
Number of Animals	8	8	8	8
Mean	0.0187	0.0131***	0.0138***	0.0146***
Range	0.0166-0.0211	0.0086-0.0161	0.0111-0.0163	0.0129-0.0180
Standard Deviation	0.0019	0.0022	0.0015	0.0017
Heart Weight				
Number of Animals	8	8	8	8
Mean	0.960	1.015	0.977	1.005
Range	0.847-1.070	0.896-1.154	0.902-1.060	0.885-1.065
Standard Deviation	0.067	0.083	0.054	0.059
Spleen Weight				
Number of Animals	8	8	8	8
Mean	0.653	0.689	0.712	0.615
Range	0.614-0.707	0.582-0.986	0.596-0.809	0.525-0.665
Standard Deviation	0.031	0.127	0.076	0.048
Adrenal Weight				
Number of Animals	8	8	8	8
Mean	0.057	0.051	0.064	0.070
Range	0.040-0.070	0.039-0.066	0.048-0.085	0.055-0.086
Standard Deviation	0.011	0.008	0.014	0.011
Brain Weight				
Number of Animals	8	8	8	8
Mean	1.938	1.946	1.934	2.029
Range	1.857-2.176	1.855-2.052	1.743-2.076	1.912-2.297
Standard Deviation	0.108	0.078	0.105	0.121
Testes Weight				
Number of Animals	8	8	8	8
Mean	3.939	4.095	3.934	4.030
Range	3.739-4.291	3.612-4.841	3.671-4.201	3.596-4.642
Standard Deviation	0.180	0.437	0.193	0.309

* significantly different from Control at p<0.05

*** significantly different from Control at p<0.001

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF BODY AND ABSOLUTE ORGAN WEIGHT MEANS (g)

29-DAY SACRIFICE (FEMALES)				
GROUP # TREATMENT	2 Control - No Treatment	4 SN-1 120 ppm	6 SN-1 470 ppm	8 SN-1 1900 ppm
Body Weight				
Number of Animals	8	8	8	8
Mean	174.8	173.6	174.4	166.9
Range	167-183	167-183	160-183	154-179
Standard Deviation	6.39	5.29	8.68	9.00
Liver Weight				
Number of Animals	8	8	8	8
Mean	4.851	4.761	4.929	5.776***
Range	4.496-5.257	4.246-5.190	4.411-5.427	5.241-6.522
Standard Deviation	0.293	0.277	0.341	0.454
Kidney Weight				
Number of Animals	8	8	8	8
Mean	1.372	1.352	1.295	1.334
Range	1.249-1.569	1.255-1.529	1.122-1.449	1.197-1.547
Standard Deviation	0.098	0.094	0.108	0.116
Pituitary Weight				
Number of Animals	8	8	8	8
Mean	0.0128	0.0109	0.0111	0.0111
Range	0.0097-0.0180	0.0094-0.0130	0.0099-0.0135	0.0089-0.0126
Standard Deviation	0.0028	0.0011	0.0013	0.0015
Thyroid Weight				
Number of Animals	8	8	8	8
Mean	0.0125	0.0117	0.0113	0.0121
Range	0.0101-0.0141	0.0095-0.0138	0.0086-0.0137	0.0105-0.0143
Standard Deviation	0.0014	0.0012	0.0016	0.0012
Heart Weight				
Number of Animals	8	8	8	8
Mean	0.660	0.700	0.691	0.651
Range	0.551-0.817	0.607-0.778	0.590-0.764	0.580-0.798
Standard Deviation	0.077	0.066	0.059	0.074
Spleen Weight				
Number of Animals	8	8	8	8
Mean	0.412	0.450	0.435	0.397
Range	0.365-0.471	0.352-0.554	0.397-0.496	0.333-0.469
Standard Deviation	0.037	0.066	0.040	0.046
Adrenal Weight				
Number of Animals	8	8	8	8
Mean	0.068	0.059	0.066	0.060
Range	0.047-0.091	0.052-0.075	0.053-0.086	0.045-0.073
Standard Deviation	0.016	0.007	0.013	0.010
Brain Weight				
Number of Animals	8	8	8	8
Mean	1.859	1.884	1.841	1.803
Range	1.693-2.015	1.794-2.066	1.716-1.925	1.706-1.908
Standard Deviation	0.095	0.094	0.074	0.063
Uterus Weight				
Number of Animals	8	8	8	8
Mean	0.496	0.406	0.541	0.479
Range	0.321-0.955	0.346-0.503	0.289-0.960	0.268-0.903
Standard Deviation	0.205	0.052	0.227	0.261
Ovary Weight				
Number of Animals	8	8	8	8
Mean	0.130	0.099**	0.101**	0.101**
Range	0.105-0.151	0.082-0.128	0.089-0.117	0.074-0.130
Standard Deviation	0.018	0.016	0.011	0.022

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF RELATIVE ORGAN WEIGHT MEANS (%)

29-DAY SACRIFICE (MALES)				
GROUP # TREATMENT	1 Control - No Treatment	3 SN-1 120 ppm	5 SN-1 470 ppm	7 SN-1 1900 ppm
Liver/Body Wt.				
Number of Animals	8	8	8	8
Mean	2.985	2.957	3.094	3.643***
Range	2.827-3.219	2.791-3.302	2.925-3.260	3.383-3.905
Standard Deviation	0.143	0.180	0.121	0.186
Kidney/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.732	0.723	0.713	0.785*
Range	0.692-0.787	0.669-0.767	0.694-0.739	0.726-0.861
Standard Deviation	0.035	0.028	0.017	0.047
Pituitary/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.0035	0.0032	0.0032	0.0035
Range	0.0028-0.0043	0.0029-0.0038	0.0029-0.0035	0.0030-0.0041
Standard Deviation	0.0005	0.0003	0.0002	0.0004
Thyroid/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.0064	0.0045***	0.0047***	0.0054*
Range	0.0058-0.0074	0.0028-0.0056	0.0038-0.0059	0.0048-0.0068
Standard Deviation	0.0006	0.0009	0.0007	0.0007
Heart/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.330	0.345	0.333	0.373**
Range	0.293-0.373	0.325-0.375	0.306-0.363	0.323-0.408
Standard Deviation	0.024	0.014	0.016	0.028
Spleen/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.224	0.234	0.243	0.228
Range	0.212-0.234	0.199-0.320	0.218-0.280	0.203-0.239
Standard Deviation	0.009	0.038	0.021	0.012
Adrenal/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.020	0.017	0.022	0.026**
Range	0.014-0.024	0.013-0.023	0.017-0.029	0.022-0.030
Standard Deviation	0.004	0.003	0.005	0.003
Brain/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.667	0.663	0.662	0.752**
Range	0.635-0.758	0.609-0.690	0.573-0.778	0.666-0.815
Standard Deviation	0.039	0.031	0.067	0.043
Testes/Body Wt.				
Number of Animals	8	8	8	8
Mean	1.354	1.392	1.343	1.493**
Range	1.265-1.402	1.275-1.572	1.179-1.424	1.426-1.694
Standard Deviation	0.049	0.107	0.088	0.089

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

*** significantly different from Control at p<0.001

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF RELATIVE ORGAN WEIGHT MEANS (%)

29-DAY SACRIFICE (FEMALES)				
GROUP # TREATMENT	2 Control - No Treatment	4 SN-1 120 ppm	6 SN-1 470 ppm	8 SN-1 1900 ppm
Liver/Body Wt.				
Number of Animals	8	8	8	8
Mean	2.775	2.743	2.828	3.460***
Range	2.614-2.920	2.454-2.904	2.653-3.198	3.214-3.644
Standard Deviation	0.119	0.149	0.177	0.161
Kidney/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.785	0.778	0.742	0.799
Range	0.737-0.857	0.725-0.836	0.681-0.812	0.717-0.864
Standard Deviation	0.046	0.038	0.044	0.042
Pituitary/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.0073	0.0063	0.0064	0.0066
Range	0.0057-0.0108	0.0056-0.0076	0.0056-0.0082	0.0054-0.0082
Standard Deviation	0.0017	0.0006	0.0009	0.0010
Thyroid/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.0071	0.0067	0.0065	0.0072
Range	0.0059-0.0081	0.0057-0.0078	0.0052-0.0075	0.0066-0.0087
Standard Deviation	0.0008	0.0007	0.0009	0.0006
Heart/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.378	0.403	0.396	0.390
Range	0.330-0.470	0.341-0.454	0.354-0.463	0.347-0.467
Standard Deviation	0.041	0.037	0.032	0.040
Spleen/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.236	0.259	0.249	0.237
Range	0.211-0.271	0.207-0.303	0.228-0.271	0.216-0.262
Standard Deviation	0.019	0.034	0.015	0.018
Adrenal/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.039	0.034	0.038	0.036
Range	0.028-0.053	0.030-0.044	0.030-0.048	0.027-0.042
Standard Deviation	0.008	0.004	0.006	0.006
Brain/Body Wt.				
Number of Animals	8	8	8	8
Mean	1.065	1.085	1.058	1.082
Range	1.005-1.207	1.008-1.167	0.962-1.167	1.025-1.132
Standard Deviation	0.070	0.050	0.066	0.039
Uterus/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.282	0.234	0.309	0.286
Range	0.186-0.522	0.200-0.296	0.181-0.545	0.166-0.564
Standard Deviation	0.108	0.033	0.125	0.154
Ovary/Body Wt.				
Number of Animals	8	8	8	8
Mean	0.075	0.057*	0.058*	0.061
Range	0.057-0.088	0.047-0.075	0.049-0.069	0.045-0.084
Standard Deviation	0.012	0.010	0.009	0.015

* significantly different from Control at p<0.05

*** significantly different from Control at p<0.001

Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF BODY AND ABSOLUTE ORGAN WEIGHT MEANS (g)

43-DAY SACRIFICE (MALES)				
GROUP # TREATMENT	1 Control - No Treatment	—	—	7 SN-1 1900 ppm
Body Weight				
Number of Animals	6	—	—	6
Mean	306.5	—	—	291.5
Range	254-326	—	—	274-306
Standard Deviation	27.10	—	—	12.42
Liver Weight				
Number of Animals	6	—	—	6
Mean	9.083	—	—	9.398
Range	7.718-9.743	—	—	8.622-10.102
Standard Deviation	0.751	—	—	0.580
Kidney Weight				
Number of Animals	6	—	—	6
Mean	2.151	—	—	2.147
Range	1.713-2.326	—	—	2.005-2.294
Standard Deviation	0.223	—	—	0.108
Pituitary Weight				
Number of Animals	6	—	—	6
Mean	0.0092	—	—	0.0096
Range	0.0067-0.0105	—	—	0.0065-0.0112
Standard Deviation	0.0015	—	—	0.0019
Thyroid Weight				
Number of Animals	6	—	—	6
Mean	0.0173	—	—	0.0153*
Range	0.0158-0.0201	—	—	0.0146-0.0165
Standard Deviation	0.0015	—	—	0.0008
Heart Weight				
Number of Animals	6	—	—	6
Mean	1.067	—	—	0.977
Range	0.915-1.337	—	—	0.940-1.011
Standard Deviation	0.148	—	—	0.026
Spleen Weight				
Number of Animals	6	—	—	6
Mean	0.689	—	—	0.622**
Range	0.647-0.745	—	—	0.578-0.644
Standard Deviation	0.033	—	—	0.025
Adrenal Weight				
Number of Animals	6	—	—	6
Mean	0.0565	—	—	0.0625
Range	0.0470-0.0690	—	—	0.0490-0.0810
Standard Deviation	0.0086	—	—	0.0130
Brain Weight				
Number of Animals	6	—	—	6
Mean	1.936	—	—	1.960
Range	1.842-1.994	—	—	1.910-2.001
Standard Deviation	0.051	—	—	0.033
Testes Weight				
Number of Animals	6	—	—	6
Mean	3.920	—	—	3.912
Range	3.791-4.023	—	—	3.750-4.015
Standard Deviation	0.099	—	—	0.106

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

The experimental mean was compared to the Control mean using Student's t-test.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF BODY AND ABSOLUTE ORGAN WEIGHT MEANS (g)

43-DAY SACRIFICE (FEMALES)				
GROUP # TREATMENT	2 Control - No Treatment	—	—	8 SN-1 1900 ppm
Body Weight				
Number of Animals	6	—	—	6
Mean	177.0	—	—	172.8
Range	166-187	—	—	164-185
Standard Deviation	8.44	—	—	8.47
Liver Weight				
Number of Animals	6	—	—	6
Mean	4.588	—	—	5.186**
Range	4.248-4.922	—	—	4.821-5.453
Standard Deviation	0.278	—	—	0.245
Kidney Weight				
Number of Animals	6	—	—	6
Mean	1.255	—	—	1.308
Range	1.138-1.350	—	—	1.276-1.344
Standard Deviation	0.079	—	—	0.026
Pituitary Weight				
Number of Animals	6	—	—	6
Mean	0.0124	—	—	0.0114
Range	0.0100-0.0144	—	—	0.0090-0.0141
Standard Deviation	0.0019	—	—	0.0017
Thyroid Weight				
Number of Animals	6	—	—	6
Mean	0.0137	—	—	0.0120*
Range	0.0115-0.0146	—	—	0.0100-0.0137
Standard Deviation	0.0011	—	—	0.0012
Heart Weight				
Number of Animals	6	—	—	6
Mean	0.611	—	—	0.659
Range	0.566-0.656	—	—	0.606-0.758
Standard Deviation	0.033	—	—	0.062
Spleen Weight				
Number of Animals	6	—	—	6
Mean	0.421	—	—	0.406
Range	0.371-0.448	—	—	0.371-0.435
Standard Deviation	0.030	—	—	0.023
Adrenal Weight				
Number of Animals	6	—	—	6
Mean	0.0626	—	—	0.0594
Range	0.0490-0.0810	—	—	0.0438-0.0842
Standard Deviation	0.0136	—	—	0.0138
Brain Weight				
Number of Animals	6	—	—	6
Mean	1.775	—	—	1.755
Range	1.712-1.830	—	—	1.642-1.799
Standard Deviation	0.045	—	—	0.059
Uterus Weight				
Number of Animals	6	—	—	6
Mean	0.407	—	—	0.463
Range	0.263-0.504	—	—	0.278-0.883
Standard Deviation	0.094	—	—	0.221
Ovary/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.0922	—	—	0.0914
Range	0.0702-0.1380	—	—	0.0687-0.1040
Standard Deviation	0.0254	—	—	0.0119

* significantly different from Control at p<0.05

** significantly different from Control at p<0.01

The experimental mean was compared to the Control mean using Student's t-test.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF RELATIVE ORGAN WEIGHT MEANS (%)

43-DAY SACRIFICE (MALES)				
GROUP # TREATMENT	1 Control - No Treatment	—	—	7 SN-1 1900 ppm
Liver/Body Wt.				
Number of Animals	6	—	—	6
Mean	2.966	—	—	3.223***
Range	2.848-3.039	—	—	3.086-3.371
Standard Deviation	0.064	—	—	0.110
Kidney/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.701	—	—	0.737*
Range	0.674-0.723	—	—	0.711-0.772
Standard Deviation	0.020	—	—	0.024
Pituitary/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.0030	—	—	0.0033
Range	0.0026-0.0034	—	—	0.0022-0.0039
Standard Deviation	0.0003	—	—	0.0006
Thyroid/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.0056	—	—	0.0053
Range	0.0052-0.0062	—	—	0.0049-0.0054
Standard Deviation	0.0005	—	—	0.0002
Heart/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.348	—	—	0.335
Range	0.309-0.413	—	—	0.316-0.343
Standard Deviation	0.038	—	—	0.011
Spleen/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.226	—	—	0.213
Range	0.202-0.265	—	—	0.209-0.218
Standard Deviation	0.022	—	—	0.004
Adrenal/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.0188	—	—	0.0213
Range	0.0146-0.0272	—	—	0.0179-0.0267
Standard Deviation	0.0047	—	—	0.0036
Brain/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.635	—	—	0.673
Range	0.595-0.725	—	—	0.635-0.702
Standard Deviation	0.047	—	—	0.028
Testes/Body Wt.				
Number of Animals	6	—	—	6
Mean	1.289	—	—	1.344
Range	1.173-1.557	—	—	1.265-1.424
Standard Deviation	0.138	—	—	0.060

* significantly different from Control at p<0.05

*** significantly different from Control at p<0.001

The experimental mean was compared to the Control mean using Student's t-test.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF RELATIVE ORGAN WEIGHT MEANS (%)

43-DAY SACRIFICE (FEMALES)				
GROUP # TREATMENT	2 Control - No Treatment	—	—	8 SN-1 1900 ppm
Liver/Body Wt.				
Number of Animals	6	—	—	6
Mean	2.592	—	—	3.003***
Range	2.446-2.675	—	—	2.843-3.164
Standard Deviation	0.080	—	—	0.129
Kidney/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.709	—	—	0.758*
Range	0.686-0.758	—	—	0.709-0.809
Standard Deviation	0.026	—	—	0.038
Pituitary/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.0070	—	—	0.0066
Range	0.0056-0.0085	—	—	0.0050-0.0086
Standard Deviation	0.0011	—	—	0.0012
Thyroid/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.0077	—	—	0.0069
Range	0.0065-0.0088	—	—	0.0060-0.0077
Standard Deviation	0.0008	—	—	0.0006
Heart/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.345	—	—	0.382
Range	0.333-0.353	—	—	0.344-0.451
Standard Deviation	0.008	—	—	0.039
Spleen/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.238	—	—	0.235
Range	0.213-0.256	—	—	0.221-0.261
Standard Deviation	0.017	—	—	0.015
Adrenal/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.0355	—	—	0.0346
Range	0.0294-0.0463	—	—	0.0265-0.0513
Standard Deviation	0.0083	—	—	0.0091
Brain/Body Wt.				
Number of Animals	6	—	—	6
Mean	1.005	—	—	1.017
Range	0.920-1.064	—	—	0.972-1.082
Standard Deviation	0.057	—	—	0.047
Uterus/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.230	—	—	0.272
Range	0.148-0.296	—	—	0.150-0.535
Standard Deviation	0.052	—	—	0.139
Ovary/Body Wt.				
Number of Animals	6	—	—	6
Mean	0.0523	—	—	0.0532
Range	0.0375-0.0789	—	—	0.0384-0.0634
Standard Deviation	0.0153	—	—	0.0086

* significantly different from Control at p<0.05

*** significantly different from Control at p<0.001

The experimental mean was compared to the Control mean using Student's t-test.

TABLE 7

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS
SUMMARY OF GROSS FINDINGS

29-DAY SACRIFICE (MALES)

	GROUP 1	GROUP 3	GROUP 5	GROUP 7
LIVER				
Discoloration			1/8 (12.50)	
URINARY BLADDER				
Concretion	1/8 (12.50)		1/8 (12.50)	2/8 (25.00)
LUNG				
Discoloration			2/8 (25.00)	
Foci			1/8 (12.50)	
Focal patches			1/8 (12.50)	
Nodules		1/8 (12.50)		
Rough texture			1/8 (12.50)	
ADRENAL GLAND				
Atrophy		1/8 (12.50)		

Group 1 = Control - No Treatment

Group 3 = SN-1 120 ppm

Group 5 = SN-1 470 ppm

Group 7 = SN-1 1900 ppm

Percentages in parentheses

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF GROSS FINDINGS

29-DAY SACRIFICE (FEMALES)				
	GROUP 2	GROUP 4	GROUP 6	GROUP 8
LIVER				
Focus		1/8 (12.50)		
URINARY BLADDER				
Concretion	1/8 (12.50)			
LUNG				
Discoloration		2/8 (25.00)	2/8 (25.00)	2/8 (25.00)
Focus		1/8 (12.50)		
Foci		1/8 (12.50)	3/8 (37.50)	2/8 (25.00)
UTERUS				
Fluid-filled				1/8 (12.50)
ADRENAL GLAND				
Atrophy				1/8 (12.50)
Hemorrhagic				1/8 (12.50)

Group 2 = Control - No Treatment
 Group 4 = SN-1 120 ppm

Group 6 = SN-1 470 ppm
 Group 8 = SN-1 1900 ppm

Percentages in parentheses

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF GROSS FINDINGS

43-DAY SACRIFICE (MALES)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	GROUP 7
URINARY BLADDER							
Concretion							1/6 (16.67)

Group 1 = Control - No Treatment

Group 7 = SN-1 1900 ppm

Percentages in parentheses

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF GROSS FINDINGS

43-DAY SACRIFICE (FEMALES)

	GROUP 2			GROUP 8
LIVER				
Discoloration	1/6 (16.67)			
Focus	1/6 (16.67)			

Group 2 = Control - No Treatment

Group 8 = SN-1 1900 ppm

Percentages in parentheses

TABLE 8

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF PCNA LABELING INDICES FOR HEPATOCELLULAR NUCLEI (%)

GROUP # TREATMENT	1 Control - No Treatment	3 SN-1 120 ppm	5 SN-1 470 ppm	7 SN-1 1900 ppm
29-DAY SACRIFICE (MALES) ¹				
Number of Animals	8	8	8	7
Mean	4.13	4.36	4.50	5.69***
Range	3.44-4.61	3.94-4.95	3.65-5.37	4.85-6.56
Standard Deviation	0.40	0.39	0.57	0.56
43-DAY SACRIFICE (MALES) ²				
Number of Animals	6	---	---	6
Mean	4.31	---	---	4.39
Range	3.70-4.98	---	---	3.17-5.55
Standard Deviation	0.58	---	---	0.95

*** significantly different from Control at p<0.001

¹ Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

² The experimental mean was compared to the Control mean using Student's t-test.

TABLE 8 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF PCNA LABELING INDICES FOR HEPATOCELLULAR NUCLEI (%)

GROUP # TREATMENT	2 Control - No Treatment	4 SN-1 120 ppm	6 SN-1 470 ppm	8 SN-1 1900 ppm
29-DAY SACRIFICE (FEMALES)¹				
Number of Animals	8	8	8	8
Mean	3.49	3.69	3.68	5.48***
Range	2.46-4.91	2.88-4.81	3.22-4.74	4.40-7.05
Standard Deviation	0.78	0.68	0.49	0.79
43-DAY SACRIFICE (FEMALES)²				
Number of Animals	6	—	—	6
Mean	4.24	—	—	5.39***
Range	3.86-4.92	—	—	4.71-5.77
Standard Deviation	0.43	—	—	0.39

*** significantly different from Control at p<0.001

¹ Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

² The experimental mean was compared to the Control mean using Student's t-test.

TABLE 9

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF PCNA LABELING INDICES FOR UROTHELIAL CELL NUCLEI (%)

GROUP # TREATMENT	1 Control - No Treatment	3 SN-1 120 ppm	5 SN-1 470 ppm	7 SN-1 1900 ppm
29-DAY SACRIFICE (MALES)¹				
Number of Animals	8	5	8	7
Mean	12.86	17.04*	15.83	18.50***
Range	9.64-18.07	13.18-20.77	13.39-18.18	14.55-21.55
Standard Deviation	2.95	3.26	1.96	2.16
43-DAY SACRIFICE (MALES)²				
Number of Animals	5	--	--	5
Mean	13.53	--	--	13.83
Range	11.72-15.81	--	--	11.52-15.38
Standard Deviation	1.67	--	--	1.53

* significantly different from Control at p<0.05

*** significantly different from Control at p<0.001

¹ Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

² The experimental mean was compared to the Control mean using Student's t-test.

TABLE 9 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF PCNA LABELING INDICES FOR UROTHELIAL CELL NUCLEI (%)

GROUP # TREATMENT	2 Control - No Treatment	4 SN-1 120 ppm	6 SN-1 470 ppm	8 SN-1 1900 ppm
29-DAY SACRIFICE (FEMALES)¹				
Number of Animals	7	8	8	8
Mean	12.59	12.89	17.91**	17.53**
Range	10.95-16.67	10.08-15.30	14.59-21.46	11.52-21.96
Standard Deviation	2.00	1.80	2.62	3.90
43-DAY SACRIFICE (FEMALES)²				
Number of Animals	6	--	--	6
Mean	10.48	--	--	14.79*
Range	8.42-15.02	--	--	10.41-17.49
Standard Deviation	2.40	--	--	2.49

* significantly different from Control at $p<0.05$

** significantly different from Control at $p<0.01$

¹ Means were compared by ANOVA followed by Dunnett's multiple comparisons test where each group is compared with the Control.

² The experimental mean was compared to the Control mean using Student's t-test.

TABLE 10

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF PERTINENT HISTOPATHOLOGY FINDINGS

29-DAY SACRIFICE (MALES)

	GROUP 1	GROUP 3 ¹	GROUP 5 ¹	GROUP 7
LIVER				
Foci of cellular alteration only				1/8 (12.50)
Foci of cellular alteration with foci of mononuclear cell infiltration	1/8 (12.50)			
Foci of mononuclear cell infiltration only	6/8 (75.00)	6/8 (75.00)	7/8 (87.50)	
Foci of mononuclear cell infiltration, including hepatocellular apoptosis				5/8 (62.50)
Foci of mononuclear cell infiltration, including hepatocellular apoptosis, with cell degeneration				2/8 (25.00)
KIDNEY				
Chronic progressive nephropathy	5/8 (62.50)			4/8 (50.00)
LUNG				
Perivascular lymphoid infiltration only	4/8 (50.00)			5/8 (62.50)
Perivascular lymphoid infiltration with aggregate of foamy histiocytes	1/8 (12.50)			
Minimal inflammatory response	1/8 (12.50)			

Group 1 = Control - No Treatment

Group 3 = SN-1 120 ppm

Group 5 = SN-1 470 ppm

Group 7 = SN-1 1900 ppm

Percentages in parentheses

¹ As per protocol, no histopathology was performed on groups 3 and 5 except for liver, heart and spleen.

TABLE 10 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF PERTINENT HISTOPATHOLOGY FINDINGS

29-DAY SACRIFICE (MALES)

	GROUP 1	GROUP 3 ¹	GROUP 5 ¹	GROUP 7
PITUITARY GLAND				
Cyst				1/8 (12.50)
THYROID				
Ultimobranchial cyst	1/8 (12.50)			
HEART				
Cardiomyopathy only	2/8 (25.00)	2/8 (25.00)		
Cardiomyopathy with cellular degeneration			3/8 (37.50)	
Cellular degeneration only		2/8 (25.00)	5/8 (62.50)	7/8 (87.50)
PANCREAS				
Focal atrophy	1/8 (12.50)			
SPLEEN				
Extramedullary hematopoiesis with pigmentation	8/8 (100.00)	8/8 (100.00)	8/8 (100.00)	8/8 (100.00)
Congestion		3/8 (37.50)	3/8 (37.50)	8/8 (100.00)
OTHER				
Forestomach hyperplasia	-			1/8 (12.50)

Group 1 = Control - No Treatment
 Group 3 = SN-1 120 ppm

Group 5 = SN-1 470 ppm
 Group 7 = SN-1 1900 ppm

Percentages in parentheses

¹ As per protocol, no histopathology was performed on groups 3 and 5 except for liver, heart and spleen.

TABLE 10 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF PERTINENT HISTOPATHOLOGY FINDINGS

29-DAY SACRIFICE (FEMALES)

	GROUP 2	GROUP 4 ¹	GROUP 6 ¹	GROUP 8
LIVER				
Focus of cellular alteration with foci of mononuclear cell infiltration			1/8 (12.50)	
Foci of mononuclear cell infiltration only	2/8 (25.00)	5/8 (62.50)	3/8 (37.50)	
Foci of mononuclear cell infiltration, including hepatocellular apoptosis				1/8 (12.50)
Foci of mononuclear cell infiltration with cytoplasmic vacuolation			1/8 (12.50)	
KIDNEY				
Chronic progressive nephropathy	2/8 (25.00)			
Mineralization	1/8 (12.50)			6/8 (75.00)
LUNG				
Perivascular lymphoid infiltration only	1/8 (12.50)			1/8 (12.50)
Perivascular lymphoid infiltration with aggregate of foamy histiocytes				3/8 (37.50)
Aggregate of foamy histiocytes with inflammatory lesions	5/8 (62.50)			
Inflammatory lesions only	1/8 (12.50)			2/8 (25.00)
Inflammatory lesions with local hemorrhage	1/8 (12.50)			
THYROID				
Ultimobranchial cyst				1/8 (12.50)

Group 2 = Control - No Treatment
 Group 4 = SN-1 120 ppm

Group 6 = SN-1 470 ppm
 Group 8 = SN-1 1900 ppm

Percentages in parentheses

¹ As per protocol, no histopathology was performed on groups 4 and 6 except for liver, heart and spleen.

TABLE 10 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF PERTINENT HISTOPATHOLOGY FINDINGS

29-DAY SACRIFICE (FEMALES)

	GROUP 2	GROUP 4 ¹	GROUP 6 ¹	GROUP 8
HEART				
Cardiomyopathy only			1/8 (12.50)	1/8 (12.50)
Cardiomyopathy with cellular degeneration			2/8 (25.00)	
Cellular degeneration only		1/8 (12.50)		5/8 (62.50)
Focus of hemorrhage	1/8 (12.50)			6/8 (75.00)
PANCREAS				
Increase of interstitial collagen with focal atrophy				1/8 (12.50)
UTERUS				
Endometrial hyperplasia	1/8 (12.50)			
VAGINA				
Squamous epithelial cyst	2/8 (25.00)			
SPLEEN				
Extramedullary hematopoiesis with pigmentation	8/8 (100.00)	8/8 (100.00)	8/8 (100.00)	8/8 (100.00)
Congestion		3/8 (37.5)	6/8 (75.00)	8/8 (100.00)
HARDERIAN GLAND				
Local inflammatory infiltrates	1/8 (12.50)			

Group 2 = Control - No Treatment
 Group 4 = SN-1 120 ppm

Group 6 = SN-1 470 ppm
 Group 8 = SN-1 1900 ppm

Percentages in parentheses

¹ As per protocol, no histopathology was performed on groups 4 and 6 except for liver, heart and spleen.

TABLE 10 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF PERTINENT HISTOPATHOLOGY FINDINGS

43-DAY SACRIFICE (MALES)

	GROUP 1			GROUP 7
LIVER				
Foci of mononuclear cell infiltration only	2/6 (33.33)			
Foci of mononuclear cell infiltration, including hepatocellular apoptosis				3/6 (50.00)
KIDNEY				
Chronic progressive nephropathy	5/6 (83.33)			6/6 (100.00)
LUNG				
Perivascular lymphoid infiltration only	1/6 (16.67)			1/6 (16.67)
Perivascular lymphoid infiltration with aggregate of foamy histiocytes	2/6 (33.33)			2/6 (33.33)
THYROID				
Ultimobranchial cyst				1/6 (16.67)
HEART				
Cardiomyopathy only	3/6 (50.00)			3/6 (50.00)
Cardiomyopathy with cellular degeneration				1/6 (16.67)
SPLEEN				
Extramedullary hematopoiesis with pigmentation	6/6 (100.00)			6/6 (100.00)
Congestion				6/6 (100.00)
MESENTERIC LYMPH NODE				
Granulomatous inflammation				2/6 (33.33)
OTHER				
Focal chronic inflammation in skeletal muscle (thigh)				1/6 (16.67)

Group 1 = Control - No Treatment

Group 7 = SN-1 1900 ppm

Percentages in parentheses

TABLE 10 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

SUMMARY OF PERTINENT HISTOPATHOLOGY FINDINGS

43-DAY SACRIFICE (FEMALES)

	GROUP 2			GROUP 8
LIVER				
Focus of cellular alteration with focal fibrosis of the portal tract	1/6 (16.67)			
Foci of mononuclear cell infiltration only	3/6 (50.00)			
Foci of mononuclear cell infiltration, including hepatocellular apoptosis				3/6 (50.00)
KIDNEY				
Chronic progressive nephropathy with mineralization	1/6 (16.67)			
Mineralization with cortical cyst				1/6 (16.67)
Mineralization only	5/6 (83.33)			4/6 (66.67)
LUNG				
Perivascular lymphoid infiltration only				1/6 (16.67)
Perivascular lymphoid infiltration with aggregate of foamy histiocytes				4/6 (66.67)
HEART				
Cardiomyopathy with myocardial fibrosis				1/6 (16.67)
PANCREAS				
Foci of cytoplasmic eosinophilia with focal atrophy				1/6 (16.67)
SPLEEN				
Extramedullary hematopoiesis with pigmentation	6/6 (100.00)			6/6 (100.00)
Congestion				6/6 (100.00)

Group 2 = Control - No Treatment

Group 8 = SN-1 1900 ppm

Percentages in parentheses

APPENDIX 1

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL BODY WEIGHTS (g)
Group 1: Control - Males

ANIMAL #	DAY						35	42
	-7	0	3	7	14	21		
1-1	195	221	234	246	269	289	307	--
1-2	200	223	234	247	271	290	308	--
2-1	200	222	233	245	265	279	291	283
2-2	201	227	239	252	270	284	297	--
3-1	203	232	242	255	272	286	300	310
3-2	203	235	246	260	278	292	302	--
4-1	206	237	247	262	281	298	317	330
4-2	206	236	244	256	273	287	308	319
5-1	207	234	248	260	276	288	309	--
5-2	211	238	246	258	275	290	309	--
6-1	213	240	256	270	287	304	320	336
6-2	214	242	252	267	286	302	316	330
7-1	216	242	252	268	286	301	321	--
7-2	219	252	260	276	291	306	326	--
MEAN	206.7	234.4	245.2	258.7	277.1	292.6	309.4	318.0
SD	7.00	8.78	8.28	9.40	7.91	8.22	9.88	19.50
								325.8
								28.27

SD = Standard Deviation

--- indicates that animal has been sacrificed

APPENDIX 1 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL BODY WEIGHTS (g)
Group 3: SN-1 120 ppm - Males

ANIMAL #	DAY						42
	-7	0	3	7	14	21	
15-1	202	227	239	251	266	279	295
15-2	203	233	238	258	284	303	324
16-1	195	223	237	255	278	293	312
16-2	199	221	228	243	264	279	295
17-1	203	225	235	249	265	280	297
17-2	210	232	243	254	270	283	305
18-1	213	240	253	267	292	306	325
18-2	218	243	255	275	299	317	336
MEAN	205.4	230.5	241.0	256.5	277.3	292.5	311.1
SD	7.65	7.96	9.09	10.23	13.32	14.66	15.74

SD = Standard Deviation

--- indicates that animal has been sacrificed

APPENDIX 1 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL BODY WEIGHTS (g)

Group 5: SN-1 470 ppm - Males

ANIMAL #	DAY						42
	-7	0	3	7	14	21	
23-1	196	216	226	235	259	272	288
23-2	198	223	234	249	270	287	307
24-1	202	221	231	244	263	280	294
24-2	203	232	243	260	284	304	326
25-1	203	237	248	262	278	296	315
25-2	210	238	248	265	284	302	321
26-1	213	234	244	265	283	297	316
26-2	218	253	265	283	307	327	347
MEAN	205.4	231.8	242.4	257.9	278.5	295.6	314.3
SD	7.60	11.73	12.22	14.88	15.05	16.79	18.56

SD = Standard Deviation

--- indicates that animal has been sacrificed

APPENDIX 1 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL BODY WEIGHTS (g)
Group 7: SN-1 1900 ppm - Males

ANIMAL #	DAY						42
	-7	0	3	7	14	21	
31-1	197	217	224	238	248	261	279
31-2	197	219	232	244	239	258	283
32-1	202	230	236	253	265	280	273
32-2	202	223	228	244	239	262	307
33-1	202	227	239	257	246	275	277
33-2	204	218	230	248	241	265	294
34-1	205	230	238	256	270	277	283
34-2	207	236	244	243	268	283	295
35-1	208	235	244	256	262	278	312
35-2	212	236	246	253	268	282	312
36-1	213	241	247	254	256	266	284
36-2	214	247	252	265	267	286	293
37-1	216	249	254	266	268	286	298
37-2	217	242	250	257	266	291	309
MEAN	206.9	232.1	240.3	252.4	257.4	275.0	292.4
SD	6.68	10.52	9.37	8.19	12.11	10.68	12.57
							299.5
							15.58
							314.3
							13.52

SD = Standard Deviation

--- indicates that animal has been sacrificed

APPENDIX 1 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL BODY WEIGHTS (g)
Group 2: Control - Females

ANIMAL #	DAY						35	42
	-7	0	3	7	14	21		
8-1	137	150	154	162	170	181	183	--
8-2	138	149	155	159	169	175	185	186
9-1	138	150	161	168	178	184	190	199
9-2	140	148	152	155	163	167	171	179
10-1	142	147	151	157	167	173	179	--
10-2	143	151	153	162	171	176	177	179
11-1	143	152	156	161	169	175	181	181
11-2	143	159	162	168	181	186	194	--
12-1	144	154	158	164	176	182	184	--
12-2	147	156	162	167	177	183	188	--
13-1	147	160	162	168	181	190	193	--
13-2	148	162	167	174	182	191	197	198
14-1	148	156	161	168	175	184	186	--
14-2	151	159	166	168	176	177	184	185
MEAN	143.5	153.8	158.6	164.4	173.9	180.3	185.1	185.3
SD	4.29	4.90	5.15	5.26	5.82	6.76	7.03	9.09
								188.2 8.77

SD = Standard Deviation

--- indicates that animal has been sacrificed

APPENDIX 1 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL BODY WEIGHTS (g)
Group 4: SN-1 120 ppm - Females

ANIMAL #	DAY						42
	-7	0	3	7	14	21	
19-1	137	146	153	159	165	176	181
19-2	138	150	155	163	170	178	181
20-1	140	151	157	160	164	179	180
20-2	141	148	154	160	167	173	181
21-1	145	153	157	160	175	183	187
21-2	148	157	159	162	171	178	181
22-1	150	160	166	171	176	187	184
22-2	153	162	166	173	180	194	196
MEAN	144.0	153.4	158.4	163.5	171.0	181.0	183.9
SD	5.90	5.76	5.07	5.42	5.66	6.76	5.41

SD = Standard Deviation

--- indicates that animal has been sacrificed

APPENDIX 1 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL BODY WEIGHTS (g)
Group 6: SN-1 470 ppm - Females

ANIMAL #	DAY						42
	-7	0	3	7	14	21	
27-1	137	145	146	154	160	167	167
27-2	137	150	153	160	172	177	179
28-1	140	148	155	162	167	177	179
28-2	141	152	159	163	179	184	190
29-1	146	158	166	169	176	184	191
29-2	148	164	167	173	180	191	197
30-1	150	161	167	171	178	178	187
30-2	153	163	166	173	186	187	199
MEAN	144.0	155.1	159.9	165.6	174.8	180.6	186.1
SD	6.09	7.30	7.94	6.93	8.19	7.46	10.63

SD = Standard Deviation

--- indicates that animal has been sacrificed

APPENDIX 1 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL BODY WEIGHTS (g)
Group 8: SN-1 1900 ppm - Females

ANIMAL #	DAY						42
	-7	0	3	7	14	21	
38-1	137	147	150	154	156	166	173
38-2	137	153	158	166	169	180	185
39-1	139	153	156	158	164	170	185
39-2	139	151	153	155	156	163	193
40-1	141	153	156	161	168	174	179
40-2	141	156	161	165	175	177	--
41-1	142	150	155	161	169	179	182
41-2	142	151	155	161	169	175	190
42-1	146	153	154	160	166	174	200
42-2	146	161	164	172	175	172	--
43-1	147	157	160	166	166	175	178
43-2	148	155	160	162	167	173	--
44-1	150	163	166	166	180	174	--
44-2	153	164	172	175	181	187	--
MEAN	143.4	154.8	158.6	163.0	168.3	174.4	187.5
SD	4.96	4.98	5.81	5.87	7.55	6.33	8.15

SD = Standard Deviation

--- indicates that animal has been sacrificed

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL FOOD CONSUMPTION DATA (g/animal/day)¹
Group 1: Control - Males

CAGE #	DAY						42
	-1	7	14	21	28	35	
1	21.4	20.2	22.6	22.4	22.1	— ³	—
2	25.9	24.9	23.1	23.8	24.1	18.4 ²	15.1
3	23.2	22.2	20.4	20.4	18.5	24.9 ²	21.7
4	27.6	23.8	21.2	22.4	23.4	22.2	21.3
5	24.9	21.8	21.1	20.5	20.3	— ³	—
6	26.8	25.8	23.3	23.3	22.5	24.3	19.9
7	25.7	26.5	23.3	23.3	23.3	— ³	—
MEAN	25.1	23.6	22.1	22.3	22.0	22.5	19.5

¹ Total food consumption per cage divided by number of animals in each cage (2 at start of study)

² Following scheduled sacrifice, each cage now contains only 1 animal from this point onward.

³ Both animals have been sacrificed.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL FOOD CONSUMPTION DATA (g/animal/day)¹
Group 3: SN-1 120 ppm - Males

CAGE #	DAY						42
	-1	7	14	21	28	35	
15	25.6	23.3	21.5	24.6	23.3	--- ²	—
16	22.6	22.5	20.4	21.5	20.8	--- ²	—
17	22.6	21.8	20.7	22.3	20.7	--- ²	—
18	24.3	23.1	21.6	21.4	22.1	--- ²	—
MEAN	23.8	22.7	21.1	22.4	21.7	---	—

¹ Total food consumption per cage divided by number of animals in each cage (2 at start of study)² Both animals have been sacrificed.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL FOOD CONSUMPTION DATA (g/animal/day)¹
 Group 5: SN-1 470 ppm - Males

CAGE #	DAY					42
	-1	7	14	21	28	
23	19.4	20.6	18.8	18.6	18.2	— ²
24	23.2	20.5	18.5	20.2	18.7	— ²
25	27.0	22.4	21.0	23.4	21.3	— ²
26	23.2	22.9	20.1	20.3	20.0	— ²
MEAN	23.2	21.6	19.6	20.6	19.6	— ²

¹ Total food consumption per cage divided by number of animals in each cage (2 at start of study)
² Both animals have been sacrificed.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL FOOD CONSUMPTION DATA (g/animal/day)¹
 Group 7: SN-1 1900 ppm - Males

CAGE #	DAY						42
	-1	7	14	21	28	35	
31	22.5	23.5	16.0	19.7	17.7	20.5 ²	22.6
32	20.6	19.3	15.2	18.1	18.1	17.5	20.6
33	18.6	21.3	11.4	19.4	18.3	--- ³	---
34	22.6	19.3	19.7	18.4	16.9	24.3 ²	25.3
35	24.2	21.0	17.7	18.8	19.3	24.4 ²	22.8
36	24.7	20.7	16.6	17.5	17.2	--- ³	---
37	22.1	21.4	19.3	20.6	20.1	27.5 ²	24.8
MEAN	22.2	20.9	16.6	18.9	18.2	22.8	23.2

¹ Total food consumption per cage divided by number of animals in each cage (2 at start of study)² Following scheduled sacrifice, each cage now contains only 1 animal from this point onward.³ Both animals have been sacrificed.

APPENDIX 2 (cont'd)

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL FOOD CONSUMPTION DATA (g/animal/day)¹
Group 2: Control - Females

CAGE #	DAY						42
	-1	7	14	21	28	35	
8	14.3	14.7	14.6	14.9	14.6 ²	14.3	
9	17.6	15.8	17.6	14.3	15.2	14.8	13.6
10	15.9	15.1	15.0	14.6	14.7	12.9 ²	14.5
11	16.8	14.9	14.4	14.3	14.5	--- ³	---
12	17.2	14.5	15.7	14.6	14.5	--- ³	---
13	13.8	14.4	13.7	14.6	15.8	14.3 ²	15.4
14	14.5	13.6	13.8	13.3	13.5	13.1 ²	13.5
MEAN	15.7	14.7	15.0	14.3	14.7	13.9	14.3

¹ Total food consumption per cage divided by number of animals in each cage (2 at start of study)² Following scheduled sacrifice, each cage now contains only 1 animal from this point onward.³ Both animals have been sacrificed.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL FOOD CONSUMPTION DATA (g/animal/day)¹
 Group 4: SN-1 120 ppm - Females

CAGE #	DAY						42
	-1	7	14	21	28	35	
19	13.8	14.4	14.4	13.4	14.7	-- ²	--
20	13.3	12.1	11.9	12.3	12.9	-- ²	--
21	16.1	14.7	15.9	15.6	14.2	-- ²	--
22	13.1	13.1	13.2	13.9	13.8	-- ²	--
MEAN	14.1	13.5	13.8	13.8	13.9	--	--

¹ Total food consumption per cage divided by number of animals in each cage (2 at start of study)

² Both animals have been sacrificed.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL FOOD CONSUMPTION DATA (g/animal/day)¹
Group 6: SN-1 470 ppm - Females

CAGE #	-1	7	14	21	DAY		35	42
					13.8	13.3		
27	15.1	13.2						
28	13.1	13.4	13.3	14.2				
29	17.2	14.6	13.3	14.8				
30	14.0	11.9	12.9	11.6				
MEAN	14.8	13.3	13.3	13.6				

¹ Total food consumption per cage divided by number of animals in each cage (2 at start of study)

² Both animals have been sacrificed.

FOUR-WEEK DIETARY STUDY OF WINGSTAY SN-1 IN FISCHER 344 RATS

INDIVIDUAL ANIMAL FOOD CONSUMPTION DATA (g/animal/day)¹
 Group 8: SN-1 1900 ppm - Females

CAGE #	DAY						42
	-1	7	14	21	28	35	
38	13.7	14.1	11.3	10.7	12.5	14.4	15.0
39	15.6	11.7	10.1	11.6	12.1	13.0 ²	15.3
40	14.4	12.1	13.1	12.3	12.5	13.4	14.3
41	17.3	14.0	12.1	12.1	12.7	--- ³	---
42	14.1	12.9	11.4	10.3	11.4	11.9 ²	13.9
43	12.9	11.9	11.3	12.2	11.9	--- ³	---
44	19.2	12.6	12.9	13.2	13.9	--- ³	---
MEAN	15.3	12.8	11.7	11.8	12.4	13.2	14.6

¹ Total food consumption per cage divided by number of animals in each cage (2 at start of study)

² Following scheduled sacrifice, each cage now contains only 1 animal from this point onward.

³ Both animals have been sacrificed.

Appendix 3
Individual Hematology Data
29-Day Sacrifice (males)



200 Girard Street, Suite 200, Gaithersburg, MD 20877
 301-921-0168 800-237-2815

Client: AMERICAN HEALTH FOUNDATION
 1 DANA RD
 VALHALLA, NY 10595
 (914) 592-2600

Date Collected: 01/22/97
 Date Received: 01/23/97
 Date Reported: 02/10/97

Client No. 1215

Study: R-1777 HEMAT

Species: RAT

Accession No.	Animal No.	Gp	Sex	Age	WBC THSN/UL	RBC MILL/UL	HGB GM/DL	HCT PERCENT	MCV FL
N 0072951	1-2	1	M		4.1	7.93	16.5	48.7	61.4
N 0072952	2-2	1	M		4.2	8.00	16.4	49.0	61.3
N 0072953	3-2	1	M		4.9	7.66	15.5	45.0	58.7
N 0072954	5-1	1	M		4.2	8.03	15.5	47.2	58.8
N 0072955	7-1	1	M		4.5	7.64	15.4	45.1	59.0
N 0072956	7-2	1	M		7.9	7.92	15.6	46.3	58.5
<hr/>				<hr/>				<hr/>	
Mean				4.97				46.88	
S.D.				1.47				59.62	
								1.35	

Group 1

N 0072957	15-1	3	M		2.9	7.75	15.4	45.6	58.8
N 0072958	15-2	3	M		5.1	7.39	15.1	44.3	60.0
N 0072959	16-2	3	M		3.5	7.04	15.2	43.9	62.4
N 0072960	17-1	3	M		5.2	7.20	15.2	44.7	62.1
N 0072961	17-2	3	M						
N 0072962	18-1	3	M		6.1	7.65	16.2	45.8	59.9
<hr/>				<hr/>				<hr/>	
Mean				4.56				44.86	
S.D.				1.32				60.64	
								1.55	

Group 3

N 0072963	23-1	5	M		4.8	8.07	16.7	49.1	60.8
N 0072964	23-2	5	M		8.5	8.25	16.4	49.5	60.0
N 0072965	24-2	5	M		2.8	7.82	16.3	47.2	60.3

Reference Range	9.4 -	6.2 -	13.4 -	40 -	52 -
	14.9	9	16.4	49	66

Appendix 3 (cont'd)
 Individual Hematology Data
 29-Day Sacrifice (males)



200 Girard Street, Suite 200, Gaithersburg, MD 20877
 301-921-0168 800-237-2815

Client: AMERICAN HEALTH FOUNDATION
 1 DANA RD
 VALHALLA, NY 10595
 (914) 592-2600

Date Collected: 01/22/97
 Date Received: 01/23/97
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Client No. 1215

Study: R-1777 HEMAT

Species: RAT

Accession No.	Animal No.	Gp	Sex	Age	WBC THSN/UL	RBC MILL/UL	HGB GM/DL	HCT PERCENT	MCV FL
N 0072966	25-1	5	M		4.5	7.81	16.4	45.9	58.8
N 0072967	25-2	5	M		6.2	7.89	16.1	47.3	60.0
N 0072968	26-1	5	M		4.9	7.86	16.3	46.1	58.6
	Mean				5.28	7.95	16.37	47.52	59.75
	S.D.				1.92	.175	.2	1.5	.87

Group 5

N 0072969	31-2	7	M		4.1	7.49	16.3	44.9	59.9
N 0072970	33-1	7	M		7.7	7.65	16.0	45.7	59.8
N 0072971	34-2	7	M		4.8	7.93	16.5	47.0	59.3
N 0072972	35-2	7	M		5.3	7.83	16.4	46.7	59.6
N 0072973	36-2	7	M		5.1	7.23	15.4	42.8	59.2
N 0072974	37-2	7	M		5.9	7.21	15.7	42.0	58.3
	Mean				5.48	7.557	16.05	44.85	59.35
	S.D.				1.24	.301	.43	2.05	.58

Group 7

Reference Range	9.4 -	6.2 -	13.4 -	40 -	52 -
	14.9	9	16.4	49	66

Appendix 3 (cont'd)
 Individual Hematology Data
 29-Day Sacrifice (males)



200 Girard Street, Suite 200, Gaithersburg, MD 20877
 301-921-0168 800-237-2815

Client: AMERICAN HEALTH FOUNDATION
 1 DANA RD
 VALHALLA, NY 10595
 (914) 592-2600

Date Collected: 01/22/97
 Date Received: 01/23/97
 Date Reported: 02/10/97

Client No. 1215

Study: R-1777 HEMAT

Species: RAT

Accession No.	Animal No.	Gp	Sex	Age	MCH PICO	MCHC GRA	PLATELET PERCENT	RDW THSN/UL	BANDS THSN/UL		
N 0072951	1-2	1	M		20.8	33.9	529	27.0	0.00		
N 0072952	2-2	1	M		20.5	33.5	617	26.9	0.00		
N 0072953	3-2	1	M		20.2	34.4	617	26.4	0.00		
N 0072954	5-1	1	M		19.3	32.8	670	26.5	0.00		
N 0072955	7-1	1	M		20.2	34.1	615	26.8	0.00		
N 0072956	7-2	1	M		19.7	33.7	610	26.5	0.00		
<hr/>				<hr/>				<hr/>			
Mean				20.12		33.73		609.7			
S.D.				.54		.55		45.4			
								.25			
<hr/>											

Group 1

N 0072957	15-1	3	M		19.9	33.8	626	27.3	0.00		
N 0072958	15-2	3	M		20.4	34.1	696	25.9	0.00		
N 0072959	16-2	3	M		21.6	34.6	708	26.1	0.00		
N 0072960	17-1	3	M		21.1	34.0	665	27.1	0.00		
N 0072961	17-2	3	M								
N 0072962	18-1	3	M		21.2	35.4	674	26.9	0.00		
<hr/>				<hr/>				<hr/>			
Mean				20.84		34.38		673.8			
S.D.				.68		.64		31.7			
								.62			
<hr/>											

Group 3

N 0072963	23-1	5	M		20.7	34.0	716	27.1	0.00
N 0072964	23-2	5	M		19.9	33.1	800	26.9	0.00
N 0072965	24-2	5	M		20.8	34.5	701	26.2	0.00

Reference Range	17.7	-	32	-	780	-	0	-	0
	19.1		33.5		1400		0		.06

Appendix 3 (cont'd)
 Individual Hematology Data
 29-Day Sacrifice (males)



200 Girard Street, Suite 200, Gaithersburg, MD 20877
 301-921-0168 800-237-2815

Client: AMERICAN HEALTH FOUNDATION
 1 DANA RD
 VALHALLA, NY 10595
 (914) 592-2600

Date Collected: 01/22/97
 Date Received: 01/23/97
 Date Reported: 02/10/97

Client No. 1215

Study: R-1777 HEMAT

Species: RAT

Accession No.	Animal No.	Gp	Sex	Age	MCH PICO	MCHC GRA	PLATELET PERCENT	RDW THSN/UL	BANDS THSN/UL
N 0072966	25-1	5	M		21.0	35.7	686	25.3	0.00
N 0072967	25-2	5	M		20.4	34.0	754	26.4	0.00
N 0072968	26-1	5	M		20.7	35.4	769	25.4	0.00
		Mean			20.58	34.45	737.7	26.22	0
		S.D.			.39	.97	43.9	.75	0

Group 5

N 0072969	31-2	7	M		21.8	36.3	766	26.6	0.00
N 0072970	33-1	7	M		20.9	35.0	763	27.3	0.00
N 0072971	34-2	7	M		20.8	35.1	938	25.5	0.00
N 0072972	35-2	7	M		20.9	35.1	792	25.7	0.00
N 0072973	36-2	7	M		21.3	36.0	787	24.4	0.00
N 0072974	37-2	7	M		21.8	37.4	881	26.7	0.00
		Mean			21.25	35.82	821.2	26.03	0
		S.D.			.46	.95	71.7	1.04	0

Group 7

Reference Range	17.7	-	32	-	780	-	0	-	0	-
	19.1				33.5		1400		0	.06

Appendix 3 (cont'd)
 Individual Hematology Data
 29-Day Sacrifice (males)



200 Girard Street, Suite 200, Gaithersburg, MD 20877
 301-921-0168 800-237-2815

Client: AMERICAN HEALTH FOUNDATION
 1 DANA RD
 VALHALLA, NY 10595
 (914) 592-2600

Date Collected: 01/22/97
 Date Received: 01/23/97
 Date Reported: 02/10/97

Client No. 1215

Study: R-1777 HEMAT

Species: RAT

Accession No.	Animal No.	Gp	Sex	Age	SEGS THSN/UL	LYMPHS THSN/UL	MONOS THSN/UL	EOSN THSN/UL	BASOS THSN/UL
N 0072951	1-2	1	M		0.70	3.36	0.04	0.00	0.00
N 0072952	2-2	1	M		0.71	3.44	0.04	0.00	0.00
N 0072953	3-2	1	M		1.13	3.68	0.10	0.00	0.00
N 0072954	5-1	1	M		0.76	3.40	0.04	0.00	0.00
N 0072955	7-1	1	M		0.95	3.42	0.09	0.05	0.00
N 0072956	7-2	1	M		1.11	6.64	0.16	0.00	0.00
<hr/>									
Mean					.893	3.99	.078	.008	0
S.D.					.197	1.303	.048	.02	0

Group 1

N 0072957	15-1	3	M		0.49	2.35	0.00	0.06	0.00
N 0072958	15-2	3	M		1.02	3.83	0.10	0.15	0.00
N 0072959	16-2	3	M		0.95	2.56	0.00	0.00	0.00
N 0072960	17-1	3	M		1.20	4.00	0.00	0.00	0.00
N 0072961	17-2	3	M						
N 0072962	18-1	3	M		1.40	4.64	0.06	0.00	0.00
<hr/>									
Mean					1.012	3.476	.032	.042	0
S.D.					.34	.983	.046	.066	0

Group 3

N 0072963	23-1	5	M		1.87	2.83	0.05	0.05	0.00
N 0072964	23-2	5	M		1.79	6.38	0.17	0.17	0.00
N 0072965	24-2	5	M		0.62	2.13	0.03	0.03	0.00
<hr/>									
Reference Range					.58 -	3.78 -	.02 -	0 -	0 -
					6.3	14.9	1.2	.31	.01

MEMORANDUM

TO: Terry O'Bryan, 8(e) Coordinator, HPVCB

THRU: Rich Hefter, Branch Chief, HPVCB 

FROM: Gino Scarano, Toxicologist, HPVCB 

DATE: August 21, 2000

RE: Review of 8EHQ-0998-14281

Recommendation

After reviewing the 28-day repeat dose study (oral route in rats at doses of 0, 120, 470, and 1900 ppm in feed) with propanoic acid, 3-(dodecylthio)-, oxbis(2,1-ethanediyoxy-2,1-ethanediyl ester (CAS No. 64253-30-1), I recommend that this test material be considered a potential high hazard concern. This conclusion is based on the following:

1. A variety of effects were observed in the animals tested.
2. A satellite group (highest tested dose) that was exposure-free for two weeks after the 28 days of exposure was used to assess recovery of effects. Many of the effects observed were reversible, but some persisted (changes in hematological parameters, relative kidney and liver weights, and microscopic damage to the spleen).
3. In females, the no-observable adverse effect level (NOAEL) was 120 ppm in feed (the lowest dose tested). There was no NOAEL in males.
4. Using the animal body weight and food consumption reported in the submission, the doses received were approximately 0, 11, 41, and 168 mg/kg (for both sexes). Effects observed at doses as low as 11 mg/kg in a 28-day study - especially ones that persisted for two weeks after exposure stopped - suggests the potential for a hazard concern.

Rationale for the Recommendation

A variety of effects, primarily hematological/clinical chemistry anomalies, were observed in treated animals. A NOAEL of 120 ppm in feed (approximately 11 mg/kg) was established in females for the following effects: changes in prothrombin time, activated partial thromboplastin time, cholesterol, iron, blood urea nitrogen, and urinary bladder proliferation index. There was no NOAEL established for males. Thus, the lowest dose tested was a lowest-observable adverse effect level (LOAEL) for dose-related decreases in relative thyroid weight, and in iron and triglycerides in blood.

The use of a satellite group at the highest dose showed that some effects persisted up to two weeks after exposure ceased: changes in certain hematological parameters, relative kidney and liver weights, and microscopic damage to the spleen.

An important uncertainty is the actual dose received by the animals. The report lists the doses as ppm in feed. Importantly, there was a decrease in food consumption during the 28-day exposure period which thus resulted in a lower actual dose of the test chemical for these exposure groups (significant decreases in the high dose groups for both sexes and the mid-dose males). Using the animal body weight and food consumption data on pages 38-41 of the submission, the estimated average doses received were 0, 11, 41, and 168 mg/kg (for both sexes). Note that this assumes average body weights and food consumption across groups - the high dose groups actually received lower doses.

The study authors concluded that the variety of effects observed at the highest dose in both sexes induces "...a reduction in intercellular glucose availability and utilization, protein catabolism, as well as inadequate absorption of iron.....(H)ypoxia in both hepatic and extrahepatic tissues.....(O)verload of the cardiovascular system...and increases in hepatocellular and urothelial cell proliferation. Although each of these alone may be considered relatively minor, taken together, along with the fact that the effects were observed after only 28 days of exposure at relatively low doses (11 or 41 mg/kg/d) warrants a more detailed look at this chemical.

If you have any questions, please do not hesitate to call me.